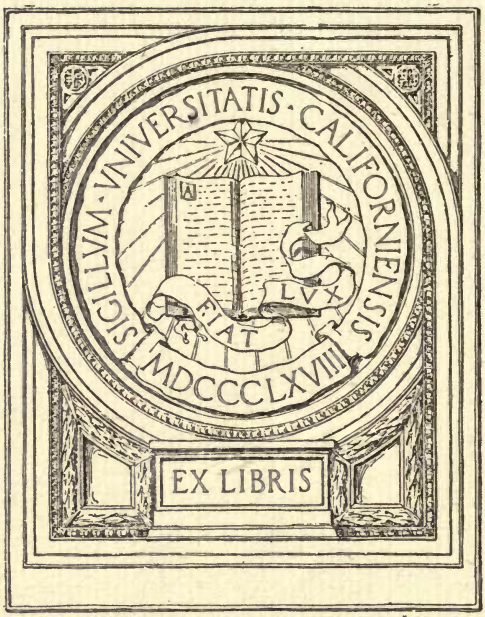
UC-NRLF
B 3 908 062



BIOLOGY
LIBRARY
G

THE METABOLISM OF BILE ACIDS

A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
AT THE UNIVERSITY OF CALIFORNIA

BY

MARJORIE GREENE FOSTER

1919

THE METABOLISM OF BILE ACIDS

I. A QUANTITATIVE METHOD FOR ANALYSIS OF BILE ACIDS IN DOG'S BILE

BY

M. G. FOSTER AND C. W. HOOPER

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY

VOL. XXXVIII, NO. 2, JUNE, 1919

QP197
F6
BIOLOGY
LIBRARY
G

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXXVII, No. 2, 1919.

THE METABOLISM OF BILE ACIDS.

I. A QUANTITATIVE METHOD FOR ANALYSIS OF BILE ACIDS IN DOG'S BILE.

BY M. G. FOSTER* AND C. W. HOOPER.

(*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.*)

(Received for publication, April 28, 1919.)

An unusual opportunity for a comprehensive study of the bile acids presented itself to us in this laboratory because of the presence of a number of bile fistula dogs under *careful routine* observation. These bile fistula dogs were under observation for the study of bile pigments. They present normal factors of weight, activity, and appetite when under the laboratory routine care which has been described in detail by Hooper and Whipple (8).

In order to study satisfactorily the metabolism of the bile acids it is necessary to have a method for the analysis of bile acids which is relatively simple and accurate and which does not require large quantities of material for analysis. The method must be specific for bile acids and react negatively with the other substances in bile and it must not be obscured by any substances which may be present in bile fistula bile. We know of no published method which meets all these requirements.

As a preliminary step we undertook to test a number of the clinical methods for the determination of bile acids, some of which are known to be inaccurate and others of which are claimed to be merely qualitative. Without exception we have found these

*This series of papers on Bile Acid Metabolism was completed just prior to the death of Miss Foster from influenza pneumonia. The work should stand as a memorial to her enthusiasm, patience, and spirit of truthful research. This work was submitted as a thesis for her degree of Doctor of Philosophy, University of California.

relatively simple clinical methods to be grossly inaccurate and useless for analysis of bile salts. This statement applies to a recent method advocated by Hoover and Blankenhorn (9). No controls are given by these workers to show that many other substances present in abnormal sera may not have been responsible for the positive bile acid reaction noted in their experiments. For a discussion of these possibilities refer to the paragraph below on Pettenkofer's test.

Some of the more elaborate chemical methods for the extraction of bile salts from bile are relatively accurate, but the large quantity of material used and the amount of time required make frequent analyses at short intervals impracticable. These extraction methods are very expensive as well as time-consuming. The older methods may be divided into three groups: (1) methods in which the bile acids are separated and weighed; (2) methods in which the bile acids are calculated from the sulfur content in whole bile; and (3) methods which depend on the color reactions of cholic acid. There are possibilities of error in all these methods, as will be pointed out later.

Methods in Which the Bile Acids are Separated and Weighed.

Huppert's Method—1864.—Huppert (11) modified Neukomm's (17) method for bile salts in urine and applied it to the bile and blood in the following way. The albumin was first coagulated with alcohol and the precipitate carefully extracted with additional amounts of alcohol. The whole extract was evaporated, dissolved in water, freed from fat by ether extraction, neutralized, and $Ba(NO_3)_2$ added to separate the fatty acids, soaps, and any remaining protein. The washed precipitate was supposedly free from bile acids. The filtrate and wash water were precipitated with lead acetate and the precipitate washed with water to free from excess acetate. The precipitate was then washed out with alcohol, heated, and Na_2CO_3 added. After it was evaporated to dryness, the residue was extracted with absolute alcohol; the alcohol was evaporated to dryness; and the residue was dissolved in water. This water solution was filtered into a weighed dish and dried to constant weight.

Socoloff's Method—1875.—Socoloff (19) published a method in which the bile acids present were calculated by the amount of bile soluble in alcohol. This work was done in Hoppe-Seyler's laboratory using a method very much like the one described in the following paragraph.

Hoppe-Seyler's Method—1881.—Hoppe-Seyler's method (10) was used by Pfaff and Balch, Stadelmann, and others. The bile was dried to constant weight at 110°C. and the residue completely extracted with boiling

absolute alcohol. The extract was allowed to stand 24 hours, filtered, and evaporated to dryness at 110°C. The residue was dissolved in absolute alcohol, evaporated to a small volume, cooled, and precipitated with a large excess of absolute ether. The bile salts which crystallized out were dried at 110°C. and weighed. Stadelmann (21) evaporated the bile to a thick syrup and allowed it to stand in contact with alcohol on the water bath some time in order to coagulate the protein and give a clear filtrate. He then repeated the extraction three times with boiling 96 per cent alcohol. He dissolved the final precipitate in water and dried to constant weight.

Croftan's Method—1902.—Croftan (3, 4) estimated the bile acids by coagulating the albumin, evaporating the filtrate and washings, and precipitating with absolute alcohol to free from salts. The filtrate was diluted with water, precipitated with basic lead acetate and ammonia, and the precipitate extracted with absolute alcohol and filtered hot. This solution of bile salts was dried to constant weight. (According to Croftan the mucin holds with it a large amount of bile acid and they are so closely bound that it is impossible to separate them even by repeated washings.)

Goodman's Method—1906.—Goodman (5) hydrolyzed 50 gm. of bile with 125 gm. of 60 per cent KOH in a reflux condenser for 24 hours. The solution was extracted five times with 75 cc. of freshly distilled petroleum ether in order to extract the cholesterol. The last of the petroleum ether was evaporated off on a water bath and the solution precipitated with 5 per cent $BaCl_2$ to separate the higher fatty acids. The precipitate was extracted with boiling water and the filtrate and washings evaporated to 200 to 300 cc. The solution was cooled in ice and salt, and acidified with HCl. After standing 2 hours the cholic acid which crystallized out was filtered off, washed, dried, and extracted in a Soxhlet apparatus with acetone. In about 5 hours all the cholic acid was dissolved and the solution was evaporated and dried to constant weight.

Methods in Which the Bile Acids Are Calculated from the Sulfur Content in Whole Bile.

Spiro's Method—1880.—The sulfur method as used by Spiro (20) consisted in fusing a definite amount of bile (usually 50 cc.) in a silver dish with KOH and KNO_3. The mixture was dissolved in water, supersaturated with HCl, and precipitated hot with $BaCl_2$. This precipitate was filtered on a paper of known ash, washed well, and dried. The main part of the precipitate was transferred to a porcelain crucible. The paper with the remainder of the precipitate was ignited and weighed. The main part of the precipitate was weighed, a few drops of concentrated H_2SO_4 added, washed out well with water into another filter paper, and this was weighed. The difference in weight showed the amount of the impurity. From this difference was calculated the $BaSO_4$ in the other portion of the precipitate which was weighed with the ash of the larger filter paper, and the total amount of sulfur was calculated from this corrected value.

Von Bergman's Method—1904.—Von Bergman (2) precipitated a 24 hour collection of bile with many volumes of 96 per cent alcohol. The mucin precipitate was filtered off and washed six or eight times with alcohol. The combined washings and filtrate were evaporated to a definite volume and an aliquot part used for the determination of the combined sulfur.

Methods Based on the Color Reactions of Cholic Acid.

Pettenkofer's Method.—This test as outlined in Hammarsten (6) is performed by dissolving a small amount of bile in concentrated sulfuric acid and warming to 60 or 70°C. A 10 per cent solution of cane sugar is added drop by drop. A beautiful red color develops which turns bluish violet on standing. The red liquid shows a spectrum with two absorption bands, one at F and one between D and E. The test fails if the solution is heated too hot, or if too much sugar is added (on account of the sugar carbonizing). Also, if impurities are present in the acid, such as H_2SO_3 or the lower oxides of nitrogen, the reaction fails. Proteins, amyl alcohol, oleic acid, morphine, etc., give a similar color so that it is necessary to carry out the spectroscopic examination also. Ville and Derrien (23) state that vanillin and anisaldehyde give the same color, and cholesterol gives a similar color. This method has been improved upon by Mylius and von Udránszky, who advise the use of a 1 per cent solution of furfurol in place of the sugar. Von Udránszky (22) emphasizes the fact that pure bile acids are necessary and suggests decolorizing the bile with charcoal and using an alcoholic solution of the residue. To each cc. of alcoholic solution, add one drop of furfurol and 1 cc. of concentrated sulfuric acid, and warm gently, This will detect $\frac{1}{20}$ to $\frac{1}{30}$ mg. of cholic acid. According to Hammarsten, the protein and fat should also be removed by neutralizing the bile and adding alcohol to at least 85 volumes per cent pure alcohol. The solution is filtered and the protein extracted with fresh alcohol, and the alcoholic extract evaporated to dryness. This residue is extracted with absolute alcohol, filtered, and the extract evaporated to dryness. The residue is extracted with ether, dissolved in water, and the solution precipitated with basic lead acetate and NH_4OH. The precipitate is washed and dissolved in boiling alcohol, filtered, and made alkaline with a few drops of NaOH. This solution is evaporated to dryness, the residue extracted with absolute alcohol, filtered, and precipitated with ether. This solution may be used for Pettenkofer's test, but even this may contain phosphatides and they give the same color reaction as do the bile acids.

Inouye and Ito (12) reported that when vanillin and concentrated sulfuric acid are added to solutions of bile acids a red line is formed at the line of contact. If the fluids are then mixed, the solution changes to a red-brown and then violet. When this solution is diluted with glacial acetic acid, an adsorption band is shown at B. This reaction is sensitive with taurocholic acid in a dilution of 1:11,000 and with cholic acid 1:22,000.

Jolles (13) published a method in which the bile acid (2 to 3 cc. of 1 per cent solution) is mixed with rhamnose (1 to 2 drops of 5 per cent solution)

and boiled with 2 to 3 cc. of concentrated HCl. A red color is produced followed by a green fluorescence which is due to the formation of methyl furfurol aldehyde. The reaction can be carried out by 0.005 to 0.0001 gm. of pure acid. It is not affected by urea, albumin, carbohydrates, hydrocarbons, or acids of the aliphatic or aromatic series, glycocoll, taurine, or cholesterol.

Authors' Method.

Dog's bile contains only taurocholic and taurocholeic acids, and on hydrolysis these split into taurine and cholic and choleic acids. Taurine is amino ethyl sulfonic acid, $CH_2NH_2CH_2HSO_3$. It acts like an α-amino-acid, and gives off its NH_2 quantitatively in 3 minutes in the Van Slyke amino nitrogen apparatus. This method consists in hydrolyzing a definite amount of bile with NaOH, thus splitting the bile acid, and then determining the amount of NH_2 in the taurine. Neither taurocholic nor taurocholeic acid gives off NH_2 before hydrolysis. In other animals both taurocholic and glycocholic acids are present, and it is therefore not certain that the method in its present form can be applied to the bile of animals other than the dog.

For the determination 5 cc. of bile cleared in the centrifuge and measured in a calibrated pipette are precipitated with 40 cc. of 95 per cent alcohol, and heated to the boiling point to insure a complete solution of the bile acid which might be held with the mucin precipitate. After cooling, the mixture is made up to 50 cc. in a cylinder with 95 per cent alcohol, and passed through a dry filter paper. Two specimens of 20 cc. each are evaporated to dryness. One is washed out with water and made up to 10 cc. in a calibrated flask. 2 cc. samples of this are used to determine the amount of NH_2 present before hydrolysis. The other is washed out quantitatively with 6 cc. of 8 per cent NaOH into a test-tube. The test-tube is loosely stoppered and placed in a boiling water bath for 5 hours. The contents are washed out into a 10 cc. calibrated flask and made up to volume with distilled water. 2 cc. samples of this are used to determine the amount of amino nitrogen due to the hydrolysis of the bile acids. The nitrogen is then figured on the basis of 1 cc. of bile for both specimens by multiplying the mg. of amino nitrogen found by 2.5 and subtracting the unhydrolyzed amino nitrogen from that due to hydrolysis. This figure is multiplied by the total volume of

bile for the 6 hour collection, which gives the total output of amino nitrogen for 6 hours. The bile acids are figured as taurocholic acid by multiplying by 36.72, the factor obtained by dividing the molecular weight of taurocholic acid by the atomic weight of nitrogen.

Quantitative Estimation of Taurine.

Table I shows that taurine gives up its NH_2 quantitatively with 3 minutes shaking in the Van Slyke amino nitrogen apparatus. The reaction is complete in that time even at as low a temperature as 13°C.

TABLE I.

Quantitative Estimation of Taurine.

Taurine.	Nitrogen gas.	Temperature.	Pressure.	Correction.	NH_2–N found.	NH_2–N theoretical.
mg.	*cc.*	*°C.*	*mm.*	*cc.*	*mg.*	*mg.*
46.00	9.11	18	760	0.10	5.157	5.152
	9.10					
9.785	2.06	22	756	0.10	1.095	1.096
	2.07					
10.58	2.17	13	759	0.14	1.189	1.185
	2.17					

Analysis of Sodium Taurocholate.

To test out the method a specimen of sodium taurocholate was analyzed for N, S, and ash, and the NH_2 yielded after hydrolysis. Table II shows that the content of both N and S of the sodium taurocholate used was about 85 per cent of their theoretical values. The 13.3 per cent ash partly accounts for the low values of N and S. Sodium taurocholate should have had only 4.3 per cent sodium in the ash if one hydrogen atom was replaced by Na.

The solutions used in Table III were hydrolyzed 5 hours in a boiling water bath and made up to 10 cc. in a calibrated flask. NH_2 determinations gave the following results.

Number.	Amount used.	Nitrogen gas.	Temperature.	Pressure.	Correction.	Amino nitrogen in 10 cc. solution.	Total NH_2—N in 10 cc.	Hydrolysis $\frac{NH_2}{N}$.
	cc.	*cc.*	*°C.*	*mm.*	*cc.*	*mg.*	*mg.*	*per cent*
1	1	1.83 1.82	20	758	0.10	9.74	10.29	94.7
2	1	1.28 1.26	20	758	0.10	6.62	6.86	96.5
3	2	1.31 1.31	20	758	0.10	3.42	3.43	99.8
4	2	0.33 0.33	20	758	0.10	0.65	0.68	95.6

TABLE II.

Analysis of Sodium Taurocholate.

Sodium taurocholate.	Total N.		Theoretical N.	Per cent of theoretical value.	Ash.
mg.	*mg.*	*per cent*	*per cent*	*per cent*	*per cent*
779.6	17.15	2.20	2.60	84.62	13.3

Sodium taurocholate.	$BaSO_4$	Total S.	Theoretical S.	Per cent of theoretical value.
mg.	*mg.*	*per cent*	*per cent*	*per cent*
250*	92.6	5.09	5.96	85.42

* The sulfur determinations were very kindly carried out by Dr. C. L. A. Schmidt.

TABLE III.

Hydrolysis of Sodium Taurocholate.

Number.	Sodium taurocholate solution.*		Water added.	Sodium taurocholate.	16 per cent of NaOH added.	NaOH in solution.
	cc.	*mg.*	*cc.*	*per cent*	*cc.*	*per cent*
1	3	467.7	0	15	3	8
2	2	311.8	1	10	3	8
3	1	155.9	2	5	3	8
4	3†	31.2	0	1	3	8

* 3.8980 gm. were made up to volume in a 25 cc. flask and definite amounts were used.

† Solution prepared by diluting 1 cc. of the original solution with 14 cc. of distilled water.

Table III shows that the hydrolysis of this specimen of sodium taurocholate in from 1 to 15 per cent solutions was approximately 100 per cent in 5 hours when the alkali present was 8 per cent during the hydrolysis.

This complete analysis was carried out on another specimen of sodium taurocholate with the same results.

Normal Constituents of Whole Bile.

According to Hoppe-Seyler (10) the normal constituents of whole bile are bile salts, bile pigments, cholesterol, mucin, ethereal sulfates, conjugated glucuronic acids, fats, soaps, a trace of urea, jercorin and other phosphatides, hydrochloric and phosphoric acids, and sulfuric acid as Na, P, Ca, Mg, Fe, and Cu salts. Marshall and Davis (16) found the same amount of urea in the bile as in the blood—32 mg. of urea per 100 cc. 1 cc. of bile would then contain 0.149 mg. of N, but Van Slyke states that only 3 per cent of N in the urea is given off in the first 3 minutes, *i.e.* 0.00447 mg. of N, and that amount is not sufficient to cause an appreciable error in this method. Traces of amino-acids have been found in disease, but these would give off their NH_2 nitrogen in the unhydrolyzed specimen, and thus would be corrected for. Stadelmann thinks that glycocholic acid may be present in dog's bile. The generally accepted opinion is that dog's bile contains no glycocholic acid or at the most a small trace. Even the presence of glycocholic acid sufficient to make up 10 per cent of the hypothetical bile acid mixture, would introduce no appreciable error, figuring all the bile acids as taurocholic acid. This is because of the large size of the cholic acid molecule and the similarity of weight of the molecules of glycocol and taurine. None of the other constituents set free NH_2 groups on hydrolysis except mucin. To obviate this error we precipitated the mucin with 10 volumes of 95 per cent alcohol. In one set it was filtered immediately; in a second, the alcohol was allowed to remain in contact with the mucin for 24 hours; and in a third, the alcoholic mixture was heated to its boiling point, cooled, and filtered immediately. Since Croftan showed that the mucin precipitate could not be freed of bile acid by extraction, we made the mixture to a definite volume with alcohol and then took an aliquot part of the filtrate. This would insure a uniform mixture of bile salts.

Table IV shows that mucin is best removed by alcohol heated to its boiling point. The NH_2 given off by the unhydrolyzed bile may be due to traces of urea or to some splitting of the bile acid. It is always proportional to the bile acid content, but not in exact ratio. The unhydrolyzed mucin-free bile gives a higher NH_2 in some cases than whole bile. This may be due to a slight splitting of the bile acid. The hydrolyzed whole bile gives off decidedly more NH_2 nitrogen than the mucin-free bile. This shows that the mucin is decomposed by hydrolysis into NH_2-

TABLE IV.

Whole Bile and Mucin-Free Bile.

Dog.	Unhydrolyzed bile.		Hydrolyzed bile.			
	Whole.	Mucin-free.	Whole.	Mucin-free.		
	NH_2—N* per cc. of bile.	NH_2—N per cc. of bile.	NH_2—N per cc. of bile.	NH_2—N per cc. of bile filtered immediately.	NH_2—N per cc. of bile after 24 hours.	NH_2—N per cc. of bile heated to boiling.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg*
17–151	0.194	0.245	1.210	0.924	1.012	0.915
16– 15	0.051	0.072	0.277	0.188	0.144	0.203
15– 22	0.205	0.245	1.386	1.185	1.214	1.276
18– 23	0.080	0.072	0.452	0.216	0.289	0.290

* The readings on the NH_2 apparatus have been omitted for simplicity.

containing substances (amino-acids). The mucin precipitated by heating the mixture of bile and alcohol to boiling seems to give the quickest and most satisfactory results.

Sodium Taurocholate Added to Bile.

To ascertain if the amino nitrogen determined in hydrolyzed bile is really specific for the bile acid, three specimens of the same bile were hydrolyzed after adding different known amounts of sodium taurocholate to each. A control was run on the bile and on the sodium taurocholate solution.

Table V shows that known amounts of sodium taurocholate added to bile can be recovered quantitatively with this method. The theoretical increase is figured from No. 1. The error is about 6 to 8 per cent loss. Since our conclusions are based on

decided increases in bile acid excretion in our experiments, a loss due to the method would have little significance in the analysis of results.

TABLE V.

Known Amounts of Sodium Taurocholate Added to Bile.

Number.	Bile.	Sodium taurocholate. (15 per cent solution).	NH_2—N increase.	NH_2—N increase due to sodium taurocholate.	Theoretical increase.	Error (loss).
	cc.	*cc.*	*mg.**	*mg.*	*mg.*	*per cent*
1	0	1		1.150		
2	5	3	4.061	3.229	3.45	6.4
3	5	2	2.953	2.121	2.30	7.8
4	5	1	1.914	1.082	1.15	6.1
5	5	0	0.832			

* The readings for the NH_2 have been omitted for simplicity.

A Six Hour Collection of Bile.

Table VI shows the same total for three periods of 2 hours each as for one period of 6 hours. This speaks for a thorough mixture of the thick viscous bile and its contained bile acids and shows that a single estimation is accurate for the total excretion. It also gives confirmatory evidence of the accuracy of this method.

TABLE VI.

Mixed Collection of Bile.

Time.	NH_2—N per 1 cc. of bile.	Volume.	Total NH_2.	
			Output.	Per 6 hours.
hrs.	*mg.*	*cc.*	*mg.*	*mg.*
1–2	1.174	15	17.61	
3–4	0.553	14	7.74	
5–6	0.558	16	8.93	34.28 Total.
1–6	0.753	45	33.88	33.88 Total.

Variations in the Concentration of Alkali.

The amount and concentration of alkali used are of minor importance as can be seen in Table VII. The same specimen of bile was hydrolyzed with varying amounts and concentration of

NaOH and the results show that the bile acid was completely hydrolyzed in all the tubes. Table VII also shows that slight variations in the amount and concentration of alkali used for the hydrolysis do not affect the accuracy of the method.

TABLE VII.

Strength of NaOH Used.

Tube.	NaOH		NH_2–N
	cc.	*per cent*	*mg.*
1	5	8	0.364
2	6	8	0.364
3	7	8	0.350
4	6	6	0.350
5	6	12	0.364

DISCUSSION.

Hammarsten (6 and 7) found jecorin, lecithin, and other phosphatides in bile, and all contain nitrogen and sulfur. Since the nitrogen is in the choline radical, it does not interfere with the present method, as choline on hydrolysis with alkalies yields trimethylamine and glycol, and trimethylamine does not react with nitrous acid. But sulfur does interfere with the bile acid methods as determined by the sulfur content.

The phosphatides cause a decided error in the methods in which the bile acids are weighed. Long and Gephart (15) have found it impossible to separate bile acids from lecithin even with acetone. Bile salts can hold in stable solution 80 per cent of their weight of egg lecithin. Part of this can be separated by precipitation, but the amount remaining with the bile acids and not separated by acetone is much in excess of that contained in any bile. Ethereal sulfates are present in some bile (human and shark) according to Hammarsten, and thus interfere with the sulfur determination. But von Bergman could not detect any in dog's bile. It is evident that there are many sources of error in all the older methods.

The method outlined in this paper is not open to the criticisms of the methods previously used for the quantitative estimation of the bile salts. It is a simple procedure requiring careful technique only in washing out the various residues and making up to volume.

The results, although 6 to 8 per cent below the theoretical values, are very constant, as we have demonstrated over and over again when duplicates were run through by several different people. The determinations can be carried out so that the results are available within 8 hours.

SUMMARY.

A method is given for the quantitative estimation of the bile acid present in dog's bile. It is based on the fact that taurine gives up its NH_2 nitrogen quantitatively in the Van Slyke amino nitrogen apparatus. The taurocholic acid is hydrolyzed by NaOH into taurine and cholic acid. The amino nitrogen of the taurine is then determined by the gasometric method.

We are indebted to both Dr. Alice Rohde and Dr. Donald D. Van Slyke for their advice and help in working out this method; also to Dr. C. L. A. Schmidt for sulfur determinations.

BIBLIOGRAPHY.

1. Beddard, A. P., and Pembrey, M. S., *Brit Med. J.*, 1902, i, 702.
2. von Bergman, G., *Beit. chem. Phys. u. Path.*, 1904, iv, 192.
3. Croftan, A. C., *Am. J. Med. Sc.*, 1902, cxxiii, 150.
4. Croftan, A. C., *Phil. Med. J.*, 1902, ix, 75, 142.
5. Goodman, E. H., *Beitr. chem. Phys. u. Path.*, 1907, ix, 91.
6. Hammarsten, O., text book of physiological chemistry, New York, 7th edition, 1914.
7. Hammarsten, O., *Ergebn. Physiol.*, 1905, iv, 7.
8. Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1916, xl, 332.
9. Hoover, C. F., and Blankenhorn, M. A., *Arch. Int. Med.*, 1916, xviii, 289.
10. Hoppe-Seyler, F., Handbuch der physiol. u. path-chem. Analyse., Berlin, 8th edition, 1909, 706; *Z. physiol. Chem.*, 1881, v, 1.
11. Huppert, H., *Arch. Heilk.*, 1864, v, 236.
12. Inouye, K., and Ito, H., *Z. physiol. Chem.*, 1908, lvii, 313.
13. Jolles, A., *Ber. chem. Ges.*, 1908, xli, 2766.
14. Kunkel, A., *Arch. ges. Physiol.*, 1877, xiv, 344.
15. Long, J. H., and Gephart, F., *J. Am. Chem. Soc.*, 1908, xxx, 1312.
16. Marshall, E. K., Jr., and Davis, D. M., *J. Biol. Chem.*, 1914, xviii, 60.
17. Neukomm, J., *Ann. Chem.*, 1860, cxvi, 38.
18. Pfaff, F., and Balch, A. W., *J. Exp. Med.*, 1897, ii, 49.
19. Socoloff, N., *Arch. ges. Physiol.*, 1875, xi, 166.
20. Spiro, P., *Arch. Physiol.*, 1880, Suppl. 50.
21. Stadelmann, E., *Z. Biol.*, 1897, xxxiv, 1.
22. von Udránszky, L., *Z. physiol. Chem.*, 1888, xii, 355.
23. Ville, J., and Derrien, E., *Compt. rend. Soc. biol.*, 1909, lxvi, 175.
24. Van Slyke, D. D., *J. Biol. Chem.*, 1913-14, xvi, 127.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE METABOLISM OF BILE ACIDS

II. NORMAL FLUCTUATIONS IN HEALTHY BILE FISTULA DOGS

BY

M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, NO. 2, JUNE, 1919

Reprinted from The Journal of Biological Chemistry, Vol. XXXVIII, No. 2, 1919

THE METABOLISM OF BILE ACIDS.

II. NORMAL FLUCTUATIONS IN HEALTHY BILE FISTULA DOGS.

By M. G. FOSTER, C. W. HOOPER, and G. H. WHIPPLE.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, April 28, 1919.)

As a preliminary to any series of bile acid experiments it is necessary to establish the normal curve of excretion. This curve of excretion may vary with the condition of the animal, with the diet administered, and with other factors which may not be subject to control. Unless otherwise noted the dogs used in these experiments were in fine physical condition as shown by normal activity, vigorous appetite, a uniform weight curve, and normal blood picture. The operative procedure and general routine care of these animals have been described in detail in an earlier publication, Hooper and Whipple (1). It is very essential that these dogs be exercised, fed, set up, and drained regularly. Diet regulation is very important and at times difficult. Diarrhea is a troublesome feature which must be controlled, and this is often best done by a proper admixture of kaolin to the food. We have found it necessary to set up the dogs for collection of bile at least 30 minutes before the actual collection is started. This assures a complete drainage of the thick viscid night bile which escapes only slowly from the fistula. The presence of this concentrated bile in the first collection will cause high readings and introduce an error unless this precaution is taken. This explains some of the high readings in the first 2 hour periods of Tables VIII and IX. Unless otherwise stated, dogs were fed exactly 2 hours after the daily collection was started and again after the period of exercise following the collection. Weights were taken in the morning before the collection.

EXPERIMENTAL.

The method of chemical analysis has been controlled and shown to be reasonably accurate. The method of bile collection has been described and may be assumed to be accurately controlled. The dogs were kept in a room in which they were under constant supervision, which assured an accurate and complete bile collection. Occasionally a dog may be restless and displace the rubber tube in the fistula allowing the escape of bile, but the binders are large and by experience carefully fitted, so that such accidents are rare. When there was loss of bile the material was discarded unless the loss was very small or could be measured. Notes are made of any deviations from the uniform routine complete collection. As explained in another place we feel that 6 or 8 hour collections are more satisfactory than the longer 12 to 24 hour collections used by Stadelmann and other workers. The longer collections are more trying to the animal and usually cause loss of weight, appetite, and strength. This immediately introduces the factor of disease with its many unknown variables. We feel that these 6 hour collections continued over weeks and months in dogs which are in every respect healthy and active will give more truthful information about the normal bile acid metabolism.

It is to be noted in the first two tables (Tables VIII and IX) that the dogs were not drained for 30 minutes before the collections were begun. The first 2 hour periods show a bile which is more concentrated and contains more bile acids than does the second unit period. A part of this high excretion during the first 2 hours is undoubtedly to be explained by the presence of some of the concentrated night bile in the bile passages, not completely drained off before collections were begun. One must keep in mind the normal fluctuations in bile acid excretion on a mixed diet so that proper care may be exercised in the interpretation of the fluctuations noted under experimental conditions. Fluctuations in bile volume are at times startling and inexplicable. The constitution of the mixed diet may account for some of the fluctuations in bile volume. It has been pointed out elsewhere by Whipple and Hooper (4) that a meat diet produces a thin, pale, voluminous bile excretion, poor in bile pigments; and further

TABLE VIII.

Bile Acid Excretion—2 Hour Periods—Mixed Diet.

Dog 18–23. Simple Bile Fistula.

Date.	Hour.	Bile.	Amino nitrogen.		Taurocholic acid output.	Weight.	Remarks.
			Per cc. of bile.	Output.			
1918		*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Mar. 5	1–2	20	0.374	7.48			Mixed diet.
	3–4	26	0.231	6.00			
	5–6	29	0.158	4.58			
	1–6	75		18.06	664	32.0	
Mar. 6	1–2	17	0.344	5.85			
	3–4	26	0.228	5.93			
	5–6	21	0.198	4.16			
	1–6	64		15.94	585	32.0	
Mar. 7	1–2	25	0.253	6.32			Hb. 110 per cent.
	3–4	28	0.170	4.76			R. B. C. 6,290,000.
	5–6	32	0.114	3.65			
	1–6	85		14.73	541	33.3	
Mar. 8	1–2	22	0.264	5.80			
	3–4	34	0.190	6.46			
	5–6	25	0.191	4.77			
	1–6	81		17.03	626	34.0	
Mar. 11	1–2	34	0.247	8.40			
	3–4	32	0.160	5.11			
	5–6	25	0.189	4.72			
	1–6	91		18.23	670	32.8	
Mar. 12	1–2	23	0.253	5.82			
	3–4	27	0.148	3.99			
	5–6	19	0.153	2.90			
	1–6	69		12.71	467	34.0	
Mar. 13	1–2	28	0.368	10.30			
	3–4	29	0.368	10.67			
	5–6	25	0.225	5.63			
	1–6	82		26.60	978	35.0	
Mar. 14	1–2	21	0.315	6.61			
	3–4	21	0.286	6.60			
	5–6	18	0.300	5.40			
	1–6	60		18.61	662	35.5	
Mar. 15	1–2	34	0.390	13.26			
	3–4	22	0.277	6.09			
	5–6	26	0.223	5.80			
	1–6	82		25.15	923	34.8	

TABLE IX.

Bile Acid Excretion—2 Hour Periods—Mixed Diet.

Dog 17–151. Simple Bile Fistula.

Date.	Hour.	Bile.	Amino nitrogen.		Taurocholic acid output.	Weight.	Remarks.
			Per cc. of bile.	Output.			
1918		*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Mar. 5	1–2	7	0.828	5.79			
	3–4	26	0.675	17.55			
	5–6	28	0.329	9.21			
	1–6	61		32.55	1,196	42.5	
Mar. 6	1–2	11	1.26	13.86			
	3–4	23	0.549	12.62			
	5–6	14	0.379	5.30			
	1–6	48		31.78	1,167	41.8	
Mar. 7	1–2	24	0.409	9.81			Hb. 118 per cent.
	3–4	25	0.267	6.67			R.B.C. 6,350,000.
	5–6	31	0.213	6.70			
	1–6	80		23.18	852	42.5	
Mar. 8	1–2	13	0.618	8.03			
	3–4	6	0.816	4.89			
	5–6	13	0.717	9.32			
	1–6	32		22.24	818	43.0	
Mar. 11	1–2	13	0.437	5.68			
	3–4	15	0.278	4.17			
	5–6	10	0.101	1.01			
	1–6	38		10.86	400	41.5	
Mar. 12	1–2	13	0.780	10.14			
	3–4	13	0.780	10.14			
	5–6	4	0.717	2.86			
	1–6	30		23.14	850	43.0	
Mar. 13	1–2	10	1.00	10.00			
	3–4	3	0.691	2.07			
	5–6	15	0.663	9.94			
	1–6	28		22.01	809	42.5	
Mar. 14	1–2	32	0.656	20.99			
	3–4 / 5–6	8	0.457	3.65			2nd and 3rd collections combined.
	1–6	40		24.64	906	43.5	
Mar. 15	1–2	13	0.717	9.32			
	3–4	5	0.654	5.88			2nd and 3rd collections combined.
	5–6	4					
	1–6	22		15.20	559	44.0	

that a carbohydrate diet is associated with a thick, scanty bile excretion, rich in bile pigments. The mixed diet used in these experiments consists of a variable mixture of kitchen scraps containing meat, bones, bread, potato, rice, soup, etc.

Table VIII shows the variations of bile salt excretion from day to day in 2 hour collections. No preliminary drainage.

Table IX shows the variation in bile acid excretion from day to day in 2 hour collections. No preliminary drainage.

Hourly Variations in Bile Acid Elimination.

Dogs were set up for 8 and 9 hours to establish the hourly curve of daily excretion of bile acids. Compare Tables X and XI with Tables VIII and IX of this paper and subsequent tables in Paper III. These experiments are characteristic of many others which need not be tabulated at this time, but some of these supplementary observations will be given in later papers to prove other points.

TABLE X.

Bile Acid Excretion—Hour Periods—Mixed Diet.

Dog 18–137. Simple Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Tauro-cholic acid per hour.	Remarks.
		Per cc. of bile.	Per hour.		
	cc.	*mg.*	*mg.*	*mg.*	
1	9.0	0.317	2.85	104	October 1.
2 and 3	20.0	0.302	3.00	110*	
4	9.0	0.274	2.47	90	
5	12.0	0.230	2.76	101	End of 5th hour fed mixed diet.
6	10.5	0.230	2.41	88	
7 and 8	22.0	0.216	2.37	87*	Hb. 90 per cent. R. B. C. 3,830,000.
9	10.5	0.230	2.41	88	Weight 24.0 lbs.

* Average of 2 hours.

Table X is quite typical of a group of experiments and shows a nearly uniform hourly elimination of bile acids. There is a general tendency for the bile acid excretion curve to fall slightly in the afternoon in spite of a midday feeding and careful preliminary drainage of the concentrated night bile before beginning the experiment.

TABLE XI.

Bile Acid Excretion—Hour Periods—Meat Diet.
Dog 17–34. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Tauro-cholic acid per hour.	Remarks.
		Per cc. of bile.	Per hour.		
	cc.	*mg.*	*mg.*	*mg.*	December 11.
1	3.8	0.686	2.61	96	Fed 500 gm. of meat.
2	9.1	0.438	3.98	147	" 300 " " "
3	9.1	0.351	3.19	118	Weight 29.8 lbs.
4	6.4	0.614	3.93	143	
5	4.1	0.614	2.52	92	
6	5.7	0.614	3.50	129	
7	5.1	0.658	3.36	124	
8	3.9	0.731	2.85	106	
9	6.0	0.686	4.10	150	

TABLE XI-a.

Bile Acid Excretion—2 Hour Periods—Fasting and Meat Diet.
Dog 17–34. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 2 hours.	Bile pigments in 2 hours.	Remarks.
		Per cc. of bile.	In 2 hours.			
	cc.	*mg.*	*mg.*	*mg.*	*mg.*	February 13.
1–2	22	0.440	9.68	355	15.3	Fasting. Mixed diet day before fasting.
3–4	20	0.332	6.64	242	13.2	
5–6	20	0.318	6.36	235	13.9	Weight 31.8 lbs.
7–8	18	0.303	5.45	201	13.1	
9–10	19	0.318	6.04	220	7.0	
February 19 to 26—Rice, potato, and milk diet.						
						February 27.
1–2	21	0.189	3.96	146	11.0	Meat 250 gm. at beginning.
3–4	16	0.132	2.11	77	8.9	
5–6	20.5	0.161	3.30	121	12.4	Weight 27.5 lbs.
7–8	14	0.247	3.46	129	11.2	
9–10	13	0.261	3.39	124	11.8	

Tables XI and XI-a show slight fluctuations in bile acid output after feeding, but we do not attach any significance to this reaction. It will be noted (Table XI-a) that the level of bile

TABLE XII.

*Bile Acid Excretion Not Influenced by "Bile Exclusion."**
Dog 18–23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
June 3	62	0.326	20.20	741	6.5	30.2	No bile exclusion.
" 4	60	0.409	24.54	902	13.6	31.2	Mixed diet.
" 5	61	0.422	25.74	932	20.9	31.2	
" 6	82	0.371	30.42	1,115	11.2		
" 7	75	0.191	14.33	526	24.8	32.2	
" 8	58	0.284	16.57	608	16.2	32.0	Hb. 127 per cent. R. B. C. 6,665,000.
" 10	75	0.224	16.80	618	22.2	30.2	
" 11	77	0.289	22.25	817	28.7	31.7	
Average.....			21.4	782			No bile exclusion.
June 12	80	0.199	15.92	584	23.7	32.0	Absolute bile exclusion.
" 13	78	Lost.			16.3	32.0	Mixed diet.
" 14	96	0.346	33.22	1,215	20.0	32.0	
" 15	72	0.248	17.86	656	30.2		
" 16							Dog set up and drained 2 hrs.
" 17	66	0.180	11.88	436	24.1	30.5	
" 18	83	0.238	19.75	725	23.3	32.0	
Average.....			19.72	723			Complete bile exclusion.

* "Bile exclusion" means total inability of the dog to lick any bile from the fistula at any time. This is effected by means of a thick gauze pad and large binder.

acid excretion is influenced by the diet of the previous day. This point will be taken up again.

This method makes it possible to follow the hourly fluctuations in the bile acid output and to establish for the first time the actual hourly curve of bile acid elimination from hour to hour.

TABLE XIII.

Bile Acid Excretion Not Influenced by "Bile Exclusion."
Dog 18–23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	No bile exclusion.
May 21	57	0.223	12.72	467	12.9	30.75	Diet 300 gm. of cracker meal, 65 gm. of meat.
" 22	52	0.255	13.26	487	10.8	30.75	Diet 300 gm. of cracker meal, 65 gm. of meat.
" 23	73	0.323	23.58	866	12.9	30.50	Diet 300 gm. of cracker meal, 65 gm. of meat.
" 24	61	0.253	15.44	567	12.9	30.25	Diet 300 gm. of cracker meal, 65 gm. of meat.
" 25–26						30.25	Mixed diet.
" 27	66					30.25	300 gm. of cracker meal, 65 gm. of meat.
" 28	72	0.221	15.90	574	7.5	30.50	300 gm. of cracker meal, 65 gm. of meat.
" 29	82	0.334	27.40	1,005	11.2	30.25	300 gm. of cracker meal, 65 gm of meat.
" 30							300 gm. of cracker meal, 65 gm. of meat.
" 31	72	0.210	15.12	552	10.0	30.00	
Average.....			17.6	645			No bile exclusion.
							Absolute bile exclusion.
June 19	77	0.252	19.40	712	18.8	32.50	300 gm. of cracker meal, 65 gm. of meat.
" 20	78	0.210	16.38	602	23.7	32.00	300 gm. of cracker meal, 65 gm. of meat.
" 21	59	0.294	17.35	637	19.8	31.50	300 gm. of cracker meal, 65 gm. of meat.
" 22	28	0.476	13.32	489	23.4	31.00	300 gm. of cracker meal, 65 gm. of meat.
Average.....			16.6	610			Complete bile exclusion.

The question of "bile exclusion" is a very important one for this entire series of experiments. The question resolves itself into the following: Does a bile fistula dog lick enough bile from

its fistula during the night to influence in any way the daily excretion of bile or bile acids? According to Stadelmann (3) in his experiments this "bile exclusion" is necessary. The exclusion of any possible ingestion of the dog's own bile in his experiments would cause a decrease of about $\frac{1}{3}$ the total bile acid excretion. Because of our respect for Stadelmann's work we felt that it was necessary to control this point beyond the peradventure of a doubt. We submit a sufficient number of experiments in Paper V to prove that under the conditions of our experiments "bile exclusion" does not influence the output of bile acids. The reasons for this are discussed more in detail in that paper. These experiments (Tables XII and XIII) make the same point and are sufficient to inform the reader that this important factor has been properly controlled in experiments that follow. Absolute "bile exclusion" does not affect the output of bile acids on either a mixed diet or a known diet. The bile salt excretion can be partly controlled by diet, as will be shown in another paper. A diet rich in meat protein increases the output and a diet poor in meat protein reduces the excretion.

Effect of Bile by Mouth on the Following Day's Excretion.

In order to find out if bile by mouth in moderate amounts affected the following day's excretion several dogs were given bile at night and the bile acid excretion followed.

Table XIV shows that bile feeding in the late afternoon does not influence the following day's excretion of bile acids. Many other experiments have been performed with identical results. It is apparent from experiments tabulated in Paper III that the greater part (about 80 per cent) of the bile acids ingested as bile will appear in the bile fistula bile within 4 hours. We may assume that only bile ingested during the early morning hours (5 to 8 a. m.) influences the bile collections in our experiments. There is no clinical evidence that the dogs lick any bile from their fistulas during this period and the experimental data confirm this point.

TABLE XIV.

Bile Feeding at Night—Mixed Diet.
Simple Bile Fistula with Splenectomy.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
17–181	Average previous 10 days.			10.60	389		
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
	July 29						4 p.m. given 58 cc. of bile by stomach tube.
	" 30	36	0.267	9.61	352	25.50	5 p.m. given 60 cc. of bile by stomach tube.
	" 31	25	0.418	10.45	384	26.25	5 p.m. given 60 cc. of bile by stomach tube.
	Aug. 1	38	0.285	10.82	397	25.00	No bile given.
	" 2	37	0.244	9.03	332	25.25	Hb. 100 per cent. R. B. C. 4,500,000.
18–137	Average previous 10 days.			14.9	547		
	July 29					22.75	4 p.m. given 58 cc. of bile by stomach tube.
	" 30	50	0.310	15.50	569	23.50	4 p.m. given 60 cc. of bile by stomach tube.
	" 31	42	0.401	16.80	617	24.0	5 p.m. given 60 cc. of bile by stomach tube.
	Aug. 1	43	0.395	16.55	608	23.5	No bile given.
	" 2	30	0.145	4.35	159	22.75	Hb. 100 per cent. R. B. C. 4,425,000.

DISCUSSION.

Loeb (2) stated that the sulfur and nitrogen excretion in the bile is higher during the first 4 hours than during the second 4 hours after eating. From the tables given in this paper it is clear that the bile acid excretion is greater during the early part of the day than later in the afternoon. There is always a tendency to fall off during the 5th and 6th hours of the collection in spite of the ingestion of food. But in an individual the amount

excreted throughout the day is fairly uniform provided a fore-period of 30 minutes or longer for drainage of the bile fistula has been a part of the routine preceding the usual 6 hour collection.

It is evident that the amount of bile which these dogs normally lick from their fistulas during the hours they spend in their cages is insufficient to cause any variation in the following day's output (Tables XII and XIII). The only time our dogs are prone to lick their fistulas is just after the completion of the daily collection. But Table XIV shows that moderate amounts of bile at this time are excreted before the following day's collection is made, and do not affect the determination in any way.

SUMMARY.

Bile acid excretion in a healthy bile fistula dog given a mixed diet will show great variations from day to day.

The amount of bile acid excreted is usually somewhat higher in the morning than in the afternoon. This holds good even after complete drainage ($\frac{1}{2}$ hour) of the concentrated night bile, and in spite of liberal feeding 2 hours after collections are begun.

The amount of bile acid excreted hour by hour during any given day is fairly uniform.

The amount of bile which a dog may lick from its fistula during the afternoon and night resting period is not sufficient in our experiments to cause any demonstrable variation in the following day's excretion.

Moderate amounts of bile given by stomach in the late afternoon do not influence the following day's excretion of bile acids.

BIBLIOGRAPHY.

1. Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1916, xl, 332.
2. Loeb, A., *Z. Biol.*, 1911, lv, 167.
3. Stadelmann, E., *Z. physiol. Chem.*, 1897, xxxiv, 1.
4. Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916, xl, 349.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE METABOLISM OF BILE ACIDS

III. ADMINISTRATION BY STOMACH OF BILE, BILE ACIDS, TAURINE, AND CHOLIC ACID TO SHOW THE INFLUENCE UPON BILE ACID ELIMINATION

BY

M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, NO. 2, JUNE, 1919

Reprinted from The Journal of Biological Chemistry, Vol. XXXVIII, No. 2, 1919.

THE METABOLISM OF BILE ACIDS.

III. ADMINISTRATION BY STOMACH OF BILE, BILE ACIDS, TAURINE, AND CHOLIC ACID TO SHOW THE INFLUENCE UPON BILE ACID ELIMINATION.

By M. G. FOSTER, C. W. HOOPER, and G. H. WHIPPLE.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, April 28, 1919.)

The cholagogue action of whole bile given by stomach is a well known fact. It has been established by many experiments that the curve of bile volume excretion does not necessarily parallel the curve of excretion of bile pigments (Whipple and Hooper, 9). In other words it is possible to stimulate a free flow of bile which is poor in bile pigments, so much so that the total bile pigment output may fall to half normal during a period of active cholagogue excretion with twice normal output of bile. Hooper (3) has pointed out several factors which may contribute to this reaction. It is well to recall that taurocholic acid given by mouth has a marked cholagogue action—experiments given below add more data to establish this statement. An ether extract of dried bile, which of course contains a mixture of substances, has no cholagogue action, but inhibits the excretion of bile pigments. It is of considerable interest to know that certain substances can stimulate or inhibit the total bile excretion while influencing the output of the various bile constituents in the same or opposite direction.

In 1875 Socoloff (7) injected glycocholic acid into a dog and found an increased excretion but no increase in the per cent of bile acids. But his method was questionable and he used only one dog. Rutherford and Vignal (5) injected bile acids into the jejunum and found an increased output of bile acids, but they do not mention how the bile acids were estimated. To Schiff (6) is given the credit for having established the fact that there is a reabsorption of bile acid and this is often termed the "circulation

of the bile." This term "circulation of the bile" is used loosely by some writers and is at times misquoted to indicate a circulation of other of the substances found in the bile, for example, bile pigments. We have definite proof that bile pigments are not absorbed from the intestine (Hooper and Whipple, 4). At present we have little if any accurate knowledge about the many other substances present in fresh bile—they may be absorbed or not.

In giving dog's bile by mouth, Stadelmann (8) found that with doses of 2.0 or 2.5 gm. of bile salt, the cholagogue action lasted up to 24 or 36 hours, but the bile salt was excreted within 24 hours, usually within 10 hours. With 1.5 gm. of pure sodium glycocholate the whole amount was excreted within 12 hours. The greatest cholagogue action was during the first 6 hours. From then on it began to abate and was over within the next 12 hours. The bile salts may have been excreted in less than 10 to 12 hours, for Stadelmann performed bile salt analyses only upon these large collections.

With doses of 3 to 5 gm. morning and evening the salts and volume were tremendously increased but the excretion was not directly proportional to the amounts given. 5 gm. twice a day for 3 successive days kept both the volume and salts above normal for more than 8 days, even though they were continually decreasing. These experiments of Stadelmann's are in harmony with those tabulated below, and supplement our experimental data.

One is not surprised to observe in the experiments given below that the curves of whole bile excretion and bile acid excretion may run parallel after the oral administration of fresh dog's bile—moreover, on the contrary, that these curves may be widely dissociated. A moderate dose of whole dog's bile given by stomach will cause a distinct cholagogue action and a parallel increase in bile acids. With the fall in bile volume after 3 to 5 hours there is a fall in the bile acid output. It is possible to give sugar with concentrated bile by stomach tube and completely inhibit the cholagogue action while a great rise in output of bile acids is taking place. We believe this procedure gives a maximum concentration of bile acids in dog's bile, as high even as 7 to 9 per cent by weight. Perhaps this represents the maximum power of the liver cells to concentrate bile acids in whole bile, at least under the conditions of the experiment.

EXPERIMENTAL.

The first four tables (Tables XV to XVIII) are to be taken as a unit because they illustrate the uniformity of reaction which follows the ingestion of moderate amounts of fresh dog's bile. The taurocholic acid content varies from 0.63 to 1.83 gm. in any given single dose. The hourly curve of bile acid excretion is remarkably

TABLE XV.

Bile Feeding.
Dog 17–181. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					August 27.
1	8.0	0.319	2.55	93	End of 1st hour given 50 cc. of whole dog's bile containing 752 mg. of taurocholic acid.
2	18.5	0.667	12.30	452	
3	11.0	0.662	7.28	267	
4	7.0	0.596	4.17	153	
5	6.5	0.333	2.16	79	End of 5th hour fed mixed diet.
6	7.0	0.222	1.55	56	
7	10.5	0.222	2.33	85	
8	10.0	0.210	2.10	77	
9	9.0	0.207	1.86	68	Hb. 100 per cent. R. B. C. 4,810,000.
10	10.0	0.221	2.21	81	Weight 25.3 lbs.

constant and usually shows that the largest elimination takes place during the first 3 hours after ingestion. Some experiments show the maximum elimination of bile acids during the first hour, again during the second or third hour. The bile acid concentration per cc. of the bile eliminated usually closely parallels the curve of total excretion. The cholagogue action is marked with the larger doses but is practically absent following the smallest dose.

Table XV shows that about 90 per cent of bile acid in whole bile given by mouth is excreted within 3 hours.

TABLE XVI.

Bile Feeding.

Dog 18–137. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					September 5.
1	10.0	0.442	4.42	162	
2	7.5	0.313	2.35	86	End of 2nd hour 75 cc. of bile containing 628 mg. of taurocholic acid.
3	11.0	0.470	5.17	189	
4	9.0	0.666	6.00	220	
5	8.5	0.696	5.92	217	End of 5th hour fed mixed diet.
6	7.0	0.464	3.25	119	
7	10.0	0.275	2.75	101	
8	8.0	0.261	2.25	82	Hb. 120 per cent. R. B. C. 5,060,000.
9	8.0	0.248	1.98	72	Weight 22.25 lbs.
10	7.0	0.221	1.55	56	

TABLE XVII.

Bile Feeding.

Dog 18–23. Simple Bile Fistula.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					August 6.
1	7.5	0.364	2.73	100	End 1st hour given 100 cc. of bile containing 1.825 gm. of taurocholic acid.
2	22.0	0.757	16.65	612	
3	17.0	0.980	16.65	612	
4	20.5	0.925	18.95	696	
5	13.5	0.582	7.86	288	End of 5th hour fed mixed diet.
6	11.0	0.373	4.10	150	
7	10.5	0.276	2.90	106	Hb. 120 per cent. R. B. C. 6,375,000.
8	9.0	0.345	3.10	114	Weight 31.5 lbs.

TABLE XVIII.

Bile Feeding.

Dog 18–137. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					September 24.
1	8.5	0.530	4.51	165	
2	11.0	0.430	4.73	173	End of 2nd hour 100 cc. of bile containing 951 mg. of taurocholic acid.
3	18.0	0.788	14.18	520	
4	16.0	0.845	13.51	499	
5	10.0	0.516	5.16	189	End of 5th hour fed mixed diet.
6	10.5	0.508	5.33	195	
7	11.0	0.326	3.58	131	
8	11.0	0.312	3.43	126	Hb. 90 per cent. R. B. C. 3,830,000.
9	10.0	0.260	2.60	95	Weight 23.3 lbs.

TABLE XIX.

Concentrated Bile Feeding.

Dog 17–34. Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					October 15.
1	7.5	0.546	4.09	150	
2	6.4	0.445	2.85	104	End 2nd hour 170 cc. of concentrated bile solution* containing 11.50 gm. of taurocholic acid given by stomach tube.
3	21.0	0.968	20.31	745	
4	26.0	1.430	37.20	1,365	Vomited about 200 cc. of fluid.
5	29.0	1.360	39.44	1,445	End 5th hour fed mixed diet.
6	34.3	1.220	41.88	1,535	
7	54.0	1.000	54.00	1,980	
8	18.0	0.657	11.82	433	Hb. 108 per cent. R. B. C. 5,700,000.
9	12.0	0.299	3.59	132	Weight 31.5 lbs.
Oct. 16	60.	0.313	18.80	690	Usual 6 hour collection.
Oct. 17	64.	0.328	21.00	752	Usual 6 hour collection.
Oct. 18	56.	0.300	16.80	616	Usual 6 hour collection.

* Solution prepared by evaporating bile to dryness, extracting with water, and centrifuging. The clear supernatant fluid is this bile solution.

Table XVI shows that about 60 per cent of bile acid administered by mouth was excreted within 4 hours. Note little if any cholagogue action.

Table XVII shows that about 90 per cent of bile acid administered by mouth was excreted in 4 hours.

Table XVIII shows that about 85 per cent of bile acid given in bile by mouth is excreted within 4 hours.

TABLE XX.

Concentrated Bile Feeding.
Dog 18–137. Simple Bile Fistula and Splenectomy.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					September 18.
1	7.5	0.187	1.40	51	
2	12.0	0.330	3.96	145	End 2nd hour 370 cc. of bile solution† containing 11.60 gm. of taurocholic acid given by stomach tube.
3	16.5	0.729	12.00	440	Vomited 60 cc.
4	20.0	1.111	22.22	816	
5	16.0	1.200	19.20	704	End 5th hour fed mixed diet.
6	14.0	1.010	14.15	519	Hb. 120 per cent. R. B. C. 5,060,000.
7, 8, and 9	45.0	0.750	11.24*	413*	Weight 23.5 lbs.

* Average of 3 hours.

† Solution prepared by evaporating bile to dryness, extracting with water, and centrifuging. The clear supernatant fluid is this bile solution.

Tables XIX and XX show remarkably well the intense reaction which may follow large doses of taurocholic acid by mouth. Whole bile was not given, but a crude watery extract of dried dog's bile which is rich in taurocholic acid. Vomiting occurred and the amount regurgitated is not known.

The cholagogue action is noted immediately and is sustained many hours and even days (Table XIX). The concentration of bile acids per cc. of bile is much above normal and the actual output of almost 2 gm. per hour is reached in one experiment. Large

amounts of bile acids seem to cause no ill effects, immediate or delayed, except some nausea and vomiting. What effect if any is produced in the general body metabolism is unknown.

Table XIX shows that bile acid fed by mouth in high concentration causes a great increase in bile acid output for 6 hours, and is a

TABLE XXI.

Concentrated Bile Feeding Plus Sugar.
Dog 15–22. Simple Bile Fistula.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 1 hour.	Remarks.
		Per cc. of bile.	In 1 hour.		
	cc.	*mg.*	*mg.*	*mg.*	
					September 26.
1	21.5	0.675	14.50	532	
2	22.0	0.380	8.36	301	End of 2nd hour 125 cc. of bile solution* containing 8.770 gm. of taurocholic acid—100 gm. of sugar-water. Little vomiting.
3	15.0	1.54	23.10	848	
4	9.5	1.93	18.35	673	
5	14.5	2.31	33.50	1,230	End of 5th hour fed mixed diet.
6	15.5	2.35	36.45	1,340	
7	15.0	2.26	33.90	1,245	
8	11.0	1.80	19.80	727	Hb. 110 per cent. R. B. C. 5,280,000.
9	8.5	1.77	15.05	552	Weight 32.8 lbs.
Sept. 27.	37.0	1.19	44.00	1,615	Usual 6 hour collection.
Sept. 30.	12.0	1.18	14.10	518	Usual 6 hour collection.

* Bile evaporated to dryness, the residue extracted with water, and centrifuged. The clear supernatant fluid is this bile solution.

cholagogue for a much longer period. About 65 per cent was excreted in 6 hours.

Table XX shows that concentrated bile acid by mouth may cause a long delayed bile acid excretion.

Table XXI shows that bile acid plus sugar by mouth causes a delayed bile acid excretion with fairly low volume of bile. The extreme cholagogue action of bile acids may be completely

inhibited by simultaneous administration of a sugar solution by stomach. Sugar solutions alone will cause the excretion of a very concentrated bile but will not modify the bile acid curve. This

TABLE XXII.

The Effect of Taurine and Cholic Acid Separately and Combined. Dog 17–151. Simple Bile Fistula.*

Date.	Volume.	Amino nitrogen. In 1 cc. of bile.	Amino nitrogen. In 6 hours.	Taurocholic acid in 6 hours.	Pigments in 6 hours.	Weight.	Urinary N.	Remarks.
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	*gm.*	
Average 11 days.	15		8.0*	294			3.3	Hb. 138 per cent. R. B. C. 7,670,000.
Mar. 30.....	34	0.277	9.42	346	26.6	36.75	2.52	Before collection: 4 gm. of cholic acid in capsule.
" 31.....	29	0.133	3.86	142	19.4	36.60	3.25	
Apr. 1.....	18	0.312	5.61	206	23.5	36.50	2.41	Before collection: 0.75 gm. of taurine in 30 cc. of salt solution intravenously.
" 2.....	28	0.353	9.89	366	17.5	36.25	2.63	
" 3.....	45	0.797	35.85	1,316	17.3	35.80	2.63	Before collection: 3 gm. of mixture ¾ cholic acid, ¼ taurine in capsule.
" 4.....	19	0.467	8.88	326	18.2	35.75	2.30	
" 5.....	47	1.141	53.60	1,967	20.9	35.50	2.58	Before collection: 4 gm. of taurocholic acid in capsule.
" 6.....	15	0.402	6.03	220		35.00	2.52	Hb. 125 per cent. R.B.C. 6,980,000.

* This dog was kept on a diet of 75 gm. of cane sugar and 100 gm. of glucose during this entire experiment, as well as during the fore-period of 11 days.

experiment gives the highest concentration of bile acids per cc. of fistula bile (86 mg. per cc.). The sugar seems responsible for a definite delay in output of bile acids; but the output is enormous in spite of the low volume and slight delay in elimination.

Tables XXII and XXIII are companion experiments and show great uniformity of reaction. From these two and many other experiments it is established that taurine by mouth or intraven-

TABLE XXIII.

The Effect of Taurine and Cholic Acid Separately and Combined. Dog 18–23. Simple Bile Fistula.*

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Pigments in 6 hours.	Weight.	Urinary N.	Remarks.
		In 1 cc. of bile.	In 6 hours.					
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	*gm.*	
Average 12 days.	24		8.7*	319			3.10	Hb. 120 per cent. R. B. C. 7,895,000.
Mar. 30.....	45	0.551	24.75	909	18.0	27.7	3.27	Before collection: 3 gm. of cholic acid in capsule.
" 31.....	34	0.183	6.22	228	24.6	27.4	3.25	
Apr. 1.....	40	0.142	5.68	208	18.8	27.1	2.57	Before collection: 0.75 gm. of taurine in 25 cc. of salt solution intravenously.
" 2.....	30	0.240	7.20	264	14.6	27.0	2.74	
" 3.....	55	0.698	38.4	1,300	10.5	26.6	2.55	Before collection: 2.6 gm. of mixture $\frac{3}{4}$ cholic acid, $\frac{1}{4}$ taurine in capsule.
" 4.....	29	0.354	10.25	376	11.1	26.5	2.69	
" 5.....	56	0.867	48.53	1,780	22.4	26.4	2.58	Before collection: 3 gm. of taurocholic acid in capsule.
" 6.....	37	0.171	6.33	232	14.8	25.8	3.30	Hb. 120 per cent. R. B. C. 7,300,000.

* This dog was kept on a diet of 75 gm. of cane sugar and 75 gm. of glucose during this entire experiment, as well as during the fore-period of 12 days.

ously does not in any way influence the output of bile acids. This is true during fasting periods as well as for full diets.

Taurocholic acid by mouth is known to be a cholagogue and this

action is well shown in our experiments. About 40 to 50 per cent of the taurocholic acid is excreted in the bile during the 6 hour collection period. There may have been slight delay in excretion owing to the use of capsules rather than solutions.

Taurine plus cholic acid fed by mouth exerts the same influence on the bile fistula dog as does the pure taurocholic acid. In other words this synthesis can take place in the body with great ease and rapidity. The cholagogue action and increased bile acid output are identical, whether the mixture of taurine plus cholic acid is given or the pure taurocholic acid.

Cholic acid alone has less than usual influence upon the bile acid output if given after a long fasting period. There may be a little increase in bile acid excretion or there may be a decided increase. The reaction seems to depend upon the amount of taurine present in the body which is available to combine with the cholic acid radical. Note additional data in Table XXIV.

Table XXIV furnishes more interesting data concerning the feeding of cholic acid and its influence upon the bile acid excretion. A small dose (2.0 gm.) of cholic acid acts as a cholagogue but does not increase the output of bile acid after an 11 day fasting period. A larger dose (4.0 gm.) of cholic acid after 9 days fasting produces a cholagogue action and a definite increase in the taurocholic acid of the bile—an increase of about 100 per cent. The next day the same dose repeated gives the same cholagogue action, but little if any increase in the bile acid output. We may assume that the first dose of cholic acid combined with all the available taurine in the body and formed taurocholic acid to be eliminated in the bile. The second large dose given the next day found no taurine to complement the cholic acid and no taurocholic acid resulted. We see therefore that either taurine or cholic acid can act as limiting factors in the over-production of taurocholic acid. This is particularly true of cholic acid, which is probably the normal determining factor. Cholic acid given by mouth during periods of full diet is usually associated with a strong cholagogue action and a great increase in output of bile acids (Table XXIV—Dog 17–34). This indicates an abundant exogenous source of taurine in the common mixed diet.

Table XXV shows the curve which results from the feeding of sodium taurocholate. Its reaction seems to be identical with that

TABLE XXIV.

Cholic Acid Feeding.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
15–22	Average.	16		10.0	367		Average 3 days after 11 days fasting.
	May 4	35	0.231	8.09	297	28.0	2.0 gm. of cholic acid in capsule at beginning.
	June 3	16	0.510	8.16	299	29.5	After 8 days fasting.
	" 4	38	0.535	20.33	746	29.0	4.0 gm. of cholic acid in emulsion at beginning.
	" 5	35	0.250	8.75	321	28.4	4.0 gm. of cholic acid in emulsion at beginning.
17–34	Oct. 9	24	0.537	12.90	473	31.5	Mixed diet.
	" 10	114	0.579	66.00	2,420	31.5	4.0 gm. of cholic acid in 15 cc. of alcohol at beginning.
	" 11	128	0.478	61.2	2,250	31.5	4.0 gm. of cholic acid in 15 cc. of alcohol at beginning.

TABLE XXV.

Bile Salt Feeding.

Dog 15–22. Simple Bile Fistula.

Hour.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Pigments in 2 hours.	Remarks.
		In 1 cc. of bile.	In 2 hours.			
	cc.	*mg.*	*mg.*	*mg.*	*mg.*	
						September 4, 1917.
1–2	18	0.800	14.14	529	6.8	End 2nd hour 10 gm. of crude sodium taurocholate given by stomach.
3–4	58	1.711	99.26	3,640	18.4	
5–6	45	1.597	71.85	2,635	11.7	
7–8	22	0.642	14.12	518	8.4	Weight 35.0 lbs.

observed after taurocholic acid feeding. The cholagogue effect is pronounced and about 50 per cent of the taurocholate is recovered in the first 4 hours of collection. The crude taurocholate was not carefully prepared or analyzed, so that the actual percentage output cannot be estimated. The reaction subsides rapidly and falls almost to normal within 6 hours.

DISCUSSION.

Von Bergman (1) fed sodium cholate to dogs and found a decided increase in the sulfur content when only 1.0 gm. was given. But his dog weighed only 4.5 kilograms. A dose of 2.0 gm. caused an even greater increase, but did not double it. He also fed a dog of 8.5 kilograms sodium cholate (2.0 gm.) every day for 3 days. The first day there was a sufficient amount of sulfur excreted to account for all the cholate being changed into taurocholic acid. But each successive day the sulfur excreted was less. The third day it was not back to normal although it was much lower. He explains this by saying that the body could not furnish sufficient taurine to unite with the abnormally high amount of sodium cholate given. Our experiments show that a minimal amount of taurine is available after a long fasting period (Table XXIV). Our mixed diet was probably richer in sulfur than von Bergman's diet of 200 gm. of meat, 150 gm. of rice, and 30 gm. of casein. Note the high output with cholic acid feeding and a liberal mixed diet (Table XXIV).

Goodman (2) gave 0.6 gm. of cholic acid to a dog on one day and found a decided increase in the amount of bile and the cholic acid excreted. This experiment was done once on a dog of 4.5 kilograms weight on a diet of dog biscuit.

The *cholic acid* appears to be the important determining factor in the output of taurocholic acid, and it is of some importance to learn the source of cholic acid in the body, its true metabolic history, and its usefulness and ultimate fate in the body. The solution of these and other questions relating to cholic acid is not easy but will repay further investigation.

Feeding cholic acid causes a minimal reaction after long periods of fasting. Repeated doses of cholic acid during fasting result in complete failure of subsequent doses to call out an increase of bile acid excretion. This may be interpreted to mean that under

these conditions of fasting and repeated ingestion of cholic acid the available taurine is reduced close to zero. The cholic acid is unable to combine with taurine in the usual way and is eliminated in some other form, perhaps in the bile or elsewhere.

Feeding cholic acid without taurine in a liberal mixed diet will give a maximal cholagogue action and output of bile acids. Presumably under these conditions there is ample taurine to combine with the cholic acid. The result is a large increase in taurocholic acid in the bile. Probably under normal conditions of diet and health there is always available an excess of taurine so that the normal determining factor is the cholic acid radical. Depending upon the available supply of cholic acid there is a high or low output of bile acids in fistula bile. It is evident that certain foods favor a high bile acid output, and presumably furnish considerable amounts of cholic acid in their metabolic history. Much more data on this point will be furnished in other publications.

SUMMARY.

When moderate amounts of bile are given by mouth (less than 1.8 gm. of taurocholic acid) about 90 per cent of the contained taurocholic acid is excreted in the first 4 hours.

Larger amounts of concentrated bile (8.0 to 11.0 gm. of taurocholic acid) may prolong the cholagogue action for many hours or even days.

A large amount of concentrated bile given with sugar causes a very high concentration of bile acids in the bile excreted (7 to 9 per cent). There may even be an absence of cholagogue action. This may represent the maximum effort of the liver cell to concentrate bile acids in bile.

Taurocholic acid and sodium taurocholate given by mouth have the familiar cholagogue action and a large amount will appear in the bile fistula bile (40 to 80 per cent) within 4 to 6 hours, depending upon the dose given.

Taurine intravenously has no effect on the excretion of bile acids.

Taurine plus cholic acid by mouth causes a marked increase in bile secretion and bile acid output—as much as does taurocholic acid itself.

Cholic acid by mouth usually causes a distinct cholagogue effect. Cholic acid fed during long periods of fasting gives a minimal output of bile acids, but fed during full diet periods gives a maximal output of bile acids. This reaction probably depends upon the available supply of taurine, which is much reduced after fasting, but is abundantly available during full diet periods.

We are indebted to Dr. C. L. A. Schmidt for a large amount of taurine which was prepared in his laboratory.

BIBLIOGRAPHY.

1. von Bergman, G., *Beitr. chem. Physiol. u. Path.*, 1904, iv, 192.
2. Goodman, E. H., *Beitr. chem. Physiol. u. Path.*, 1907, ix, 91.
3. Hooper, C. W., *Am. J. Physiol.*, 1917, xlii, 280.
4. Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1917, xlii, 264.
5. Rutherford, W., and Vignal, M., *J. Anat. and Physiol.*, 1876, x, 253; *ibid.*, 1877, xi, 61, 623.
6. Schiff, M., *Arch. ges. Physiol.*, 1870, iii, 598.
7. Socoloff, N., *Arch. ges. Physiol.*, 1875, xi, 166.
8. Stadelmann, E., *Z. physiol. Chem.*, 1897, xxxiv, 1.
9. Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916, xl, 349.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE METABOLISM OF BILE ACIDS

IV. ENDOGENOUS AND EXOGENOUS FACTORS

BY

M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, NO. 2, JUNE, 1919

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXXVIII, No. 2, 1919.

THE METABOLISM OF BILE ACIDS.

IV. ENDOGENOUS AND EXOGENOUS FACTORS.

BY M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, April 28, 1919.)

This paper gives the results of experiments to show the excretion of bile acids during periods of fasting, of sugar feeding, and of standard diets. The total daily urinary nitrogen excretion is given in some experiments to show that the output of bile acids runs an interesting parallel to the body's endogenous nitrogen metabolism. Limited diets may profoundly influence the curve of bile acid excretion, and the nitrogenous portion of the diet is most important. It is obvious that certain meat proteins added to the diet profoundly modify the excretion of bile acids. So there seems to be an interesting relationship between the metabolism of meat proteins from the food and of bile acids excreted in the bile. It is at least possible that a similar relationship may hold for the tissue proteins of the body and the bile acid excretion. Much more experimental data must be submitted but the experiments outlined below make certain fundamental points quite clear.

Bidder and Schmidt (1) followed the solid constituents of the bile and concluded that there was an increase on a meat diet. Spiro (3) followed the sulfur in the food and in the bile and found that increasing amounts of meat increased the sulfur excreted, but not proportionately. He also stated that there was a continuous sulfur excretion during fasting, but at a much lower level than when fed. Also, feeding carbohydrates decreases the amount of sulfur excreted. These experiments are in accord with our results.

Kunkel (2) followed the sulfur partition in a single day and found that the sulfur increased in the bile on starvation at one time, and not at another. Bread and milk decreased the sulfur while various amounts of meat did not have any effect at all. Twice that amount of blood by stomach decreased the bile sulfur. The dog lived only about a month after the experiment, so that very little importance can be attached to these experiments.

EXPERIMENTAL.

These dogs were kept on the usual routine already described for the previous articles of this series. The experiments given in Tables XXXI to XXXIV show the urinary nitrogen excretion

TABLE XXXI.

Sugar Feeding.
Dog 17–151. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Urinary N in 24 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.					
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*lbs.*	
Mar. 18	13	0.815	10.59	389	38.4		43.0	75 gm. of cane sugar, 50 gm. of glucose given daily by stomach tube.
" 19	16	0.618	9.88	363	69.5	4.76	40.7	Hb. 125 per cent. R. B. C. 6,840,000.
" 20	11	0.972	10.69	392	42.0	3.05	40.8	
" 21	10	0.802	8.02	294	20.9	3.16	39.7	No bile exclusion in this experiment.
" 22						3.89	38.7	
" 23	13	0.780	10.14	372	36.6	2.71	39.0	75 gm. of cane sugar, 100 gm. of glucose given daily by stomach tube.
" 24	12	0.482	5.78	212	37.6	3.33	38.4	
" 25	11	0.556	6.11	224	21.0	3.92	38.5	
" 26	15	0.379	5.68	209	26.1	3.42	38.0	
" 27	30	0.236	7.08	260	14.6	3.41	37.9	
" 28	20	0.279	5.58	205	29.2	3.17	37.25	Hb. 138 per cent. R. B. C. 7,670,000.
" 29	19	0.472	8.98	329	30.8	3.11	37.0	
Average.....			8.05	295		3.45		86 mg. of bile acid per 1.0 gm. of urinary nitrogen.

per 24 hour periods. In these experiments the dogs were kept at all times in standard metabolism cages arranged for complete collection of the urine with elimination of the feces. Diarrhea was never present and no fecal nitrogen is included in these figures. The night bile of course was included in urine collections, but the nitrogen concerned is constant and rarely exceeds 0.2 gm.

per 24 hours. The dogs were catheterized at the same hour each day, weighed, and given the sugar solutions or water by stomach tube. The cage was then washed out and the washings added to the cage urine, bladder urine, and bladder washings, which were made up to a unit volume. Duplicate specimens were analyzed by the Kjeldahl method and the total nitrogen calcu-

TABLE XXXII.

Fasting.

Dog 17–151. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Urinary N in 24 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.					
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*lbs.*	
April 23	38	0.513	19.50	716	13.2		40.7	No bile exclusion in this experiment.
" 24	18	0.510	9.18	337	41.3	2.24	38.6	Hb. 132 per cent. R. B. C. 6,800,000.
" 25	15	0.704	10.56	388	33.9	4.14	37.4	
" 26	15	0.508	7.62	279	24.9	3.97	36.6	
" 27	11	0.615	6.76	248	15.2	4.20	36.0	Set up 3 hours.
" 28	27				16.0	4.62	34.5	" " 3 "
" 29	25	0.543	13.68	502	13.1	4.20	35.0	
" 30	15	0.641	9.61	352	12.0	3.83	34.5	
May 1	18	0.711	12.78	468	12.5	3.55	34.13	
" 2	16	0.899	14.39	528	9.5	3.78	33.56	
" 3	15	0.676	10.15	373	11.8	3.97	33.13	Hb. 130 per cent. R. B. C. 7,235,000.
Average.....			11.42	419		3.85		109 mg. of bile acid per 1.0 gm. of urinary nitrogen.

lated. One of the dogs (15–22, Table XXXIV) has an obstruction in his urethra which makes catherization impossible. The regular 24 hour collections were made as usual, and the average of several days will correct for the daily variations which are in part due to variable amounts of bladder urine retained on different days.

Tables XXXI and XXXII are to be compared, as these experiments were performed upon the same healthy, vigorous dog under

identical experimental conditions. The amount of sugar given in the first experiment was not large for a dog of this size, and was increased from 125 gm. per day to 175 gm. during the last half of the experiment. It may be merely a coincidence, but during the second period of higher sugar intake the bile acid output fell somewhat. This dog averaged 3.45 gm. of urinary nitrogen per day and 295 mg. of taurocholic acid per 6 hours. The 6 hour amount of taurocholic acid per gm. of daily nitrogen is therefore 86 mg.

When this dog is put on fasting after a suitable resting period with liberal mixed diet we note a higher urinary nitrogen output, 3.85 gm. per day and 419 mg. of taurocholic acid per 6 hours. The amount per gm. of 24 hour nitrogen is therefore 109 mg. taurocholic acid per 6 hour period. As the endogenous urinary nitrogen excretion rises with fasting, as compared with sugar feeding, we note a parallel or slightly greater rise in the output of taurocholic acid.

We wish to point out an interesting fact in Tables XXXII and XXXIV. The first day in each table gives a remarkably high taurocholic acid figure, and this may not be clear until one remembers that a full mixed diet preceded this fasting period. The first day's bile acid output results from the mixed diet of the preceding day, but it is significant that after the first 24 hours the base line is reached and maintained. This is somewhat different from the basal nitrogen excretion, which does not reach its lowest level on fasting until the third or fourth day.

Tables XXXIII and XXXIV are like the preceding two experiments and correspond in almost every detail. These two experiments were done on two different dogs of approximately the same weight. Both were in excellent condition. The fasting dog of course shows a higher output of urinary nitrogen and also of taurocholic acid per 6 hour period. The fasting dog shows a urinary nitrogen of 3.76 gm. per 24 hours and taurocholic acid 407 mg. per 6 hours. The sugar fed dog presents a urinary nitrogen of 3.23 gm. per 24 hours and taurocholic acid 318 mg. per 6 hours. The amount of 6 hour taurocholic acid per gm. of 24 hour nitrogen is almost identical, 108 mg. in fasting and 98 mg. with sugar.

It seems sufficiently clear that there is a close relationship

between the endogenous nitrogen metabolism and the excretion of taurocholic acid in bile fistula bile. During fasting periods we may assume that more body protein is broken down and more taurocholic acid results from this process. We may assume that

TABLE XXXIII.

Sugar Feeding.
Dog 18–23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Urinary N in 24 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.					
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*lbs.*	
Mar. 18	52	0.204	10.60	389	26.2		33.3	75 gm. of cane sugar, 50 gm. of glucose given daily by stomach tube.
" 19	27	0.329	8.88	326	17.4	4.51	31.75	Hb. 118 per cent. R. B. C. 6,130,000.
" 20	31	0.324	10.04	368	15.6	3.72	31.06	No bile exclusion in this experiment.
" 21	17	0.507	8.62	316	10.1	3.08	30.75	
" 22	20	0.414	8.28	304	13.9	3.88	29.19	
" 23	22	0.288	6.33	232	10.7	3.22	29.75	75 gm. of cane sugar, 75 gm. of glucose given daily by stomach tube.
" 24	17	0.512	8.70	319	14.2	2.04	29.56	
" 25	23	0.394	9.06	333	22.0	3.64	29.44	
" 26	26	0.325	8.45	310	11.3	3.13	28.0	
" 27	33	0.194	6.40	235	12.6	3.19	28.56	
" 28	20	0.363	7.26	266	10.4	2.74	27.38	Hb. 120 per cent. R. B. C. 7,895,000.
" 29	34	0.332	11.30	415	14.2	2.38	27.06	
Average.....			8.66	318		3.23		98 mg. of bile acid per 1.0 gm. of urinary nitrogen.

sugar feeding enables the body to conserve its protein at the source, or enables the body to conserve its protein end-products and reconstruct these into body cells. When less bile acids are excreted during sugar periods we may wish to assume some such conservation of bile acid or its parent substance for other uses in

body metabolism. Refer also to Table XL, where there is even more conservation of bile acids or substances from which they are derived.

Fasting does not decrease the bile acid output to a remarkably low level. Sugar feeding causes a drop of bile acid excretion below the fasting level. These two points emphasize the fact

TABLE XXXIV.

Fasting.

Dog 15–22. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Urinary N in 24 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.					
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*lbs.*	
April 23	50	0.668	33.40*	1,225*	21.8		34.7	Dog not catheterized during experiment.
" 24	20	0.602	12.04	442	20.9	5.71	33.0	Hb. 150 per cent. R. B. C. 6,390,000.
" 25	27	0.470	12.68	465	32.9	3.75	32.38	No bile exclusion in this experiment.
" 26	18	0.486	8.77	322	21.9	3.16	31.5	
" 27	28	0.536	15.00	550	4.4	2.91	31.4	Set up 3 hours.
" 28	27	0.552	14.90	547	10.3	4.59	30.56	" " 3 "
" 29	22	0.493	10.85	398	4.2	3.58	30.20	
" 30	20	0.404	8.09	297	22.6	2.94	29.63	
May 1	17	0.505	8.58	315	26.6	3.19	29.20	
" 2	12	0.744	8.93	328	16.3	4.03	28.70	
Average.....			11.09	407		3.76		108 mg. of bile acid per 1.0 gm. of urinary nitrogen.

* Not included in average.

that there is an important endogenous factor in the bile acid metabolism.

Tables XXXV and XXXVI are identical in practically every respect and show a remarkable parallelism between the food intake nitrogen and the output of taurocholic acid. It will be shown later that this reaction depends in part upon the type of food protein. The first dog (Table XXXV) was in perfect condition during the entire experiment, maintained a constant weight,

TABLE XXXV.

Nitrogen in Food and Bile Acid Excretion.
Dog 17–151. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Jan. 7	43	0.403	17.32	636	21.4	38.0	Diet 375 gm. of cracker meal, 90 gm. of beef heart containing 100 calories and 0.5 gm. of nitrogen per kilo.
" 8	30	0.575	17.25	633	23.0	38.0	Hb. 135 per cent. R. B. C. 7,576,000.
" 9	49	0.246	12.05	442	24.7	38.0	
" 10	32	0.505	16.16	593	10.6	38.5	
" 11	28	0.311	8.70	319	6.3	38.5	
" 14	20	0.680	13.60	499	20.8	38.5	
" 15	65	0.260	16.90	620	20.1	39.0	No bile exclusion in this experiment.
" 16	28	0.766	21.44	787	18.5	39.0	
" 17	37	0.538	19.90	730	19.9	39.3	
" 18	17	0.785	13.35	490	16.0	38.8	Diarrhea.
" 21	16	0.604	9.66	354	18.9	39.0	
" 22	23	0.591	13.59	499	18.4	39.0	
Average.....			14.99	550	18.2		Diet 0.5 gm. of nitrogen per kilo.
Jan. 23	67	0.415	27.80	1,020	23.6	39.2	696 gm. of beef heart, 100 calories and 1.0 gm. of nitrogen per kilo.
" 24	50	0.460	27.00	994	14.8	39.5	
" 25	21	0.500	10.50	386	1.8	39.0	
" 28	37	0.675	24.97	918	3.9	39.3	
" 29	25	1.016	25.40	934	1.7	39.0	
" 30	32	0.898	28.73	1,055	2.8	39.3	
" 31	37	0.733	27.12	998	7.7	39.0	
Feb. 1	44	0.729	32.09	1,177	20.7	39.0	
" 4	51	0.645	32.89	1,206	8.0	39.0	
" 6	18	1.083	19.49	715	27.6	39.0	
" 7	40	0.871	34.84	1,279	7.7	38.8	
" 8	35	0.870	30.45	1,118	10.4	39.0	
Average.....			26.77	982	10.9		Diet 1.0 gm. of nitrogen per kilo.

TABLE XXXVI.

Nitrogen in Food and Bile Acid Excretion.
Dog 18–23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Jan. 7	42	0.272	11.02	405	40.9	33.0	Diet 325 gm. of cracker meal, 80 gm. of beef heart containing 0.5 gm. of nitrogen and 100 calories per kilo.
" 8	62	0.158	9.79	359	27.0	33.3	
" 9	72	0.073	5.22	191	25.0	33.0	Hb. 115 per cent. R. B. C. 7,024,000.
" 10	67	0.206	13.80	506	29.4	33.0	
" 11	69	0.158	10.93	401	17.6	32.8	No bile exclusion in this experiment.
" 14	67	0.143	9.58	453	20.8	33.0	
" 15	76	0.088	6.68	245	26.4	33.0	
" 16	56	0.211	11.81	433	14.6	33.5	
" 17	74	0.057	4.21	154	18.1	33.1	
" 18	70	0.184	12.85	472	12.2	33.3	
" 21	75	0.202	15.13	555	18.3	33.5	
" 22	68	0.226	15.40	566	19.9	33.5	
Average.....			10.53	395	22.5		Diet 0.5 gm. of nitrogen per kilo.
Jan. 23	79	0.3431	27.1	994	15.2	33.8	602 gm. of beef heart, 100 calories and 1.0 gm. of nitrogen per kilo.
" 24	77	0.246	18.93	685	18.8	33.6	
" 25	82	0.217	17.77	552	16.7	33.0	
" 28	43	0.392	16.85	618	11.0	32.0	350 gm. not eaten. Diarrhea.
" 29	58	0.416	24.12	885	9.1	31.8	250 " " " "
" 30	55	0.489	26.89	986	22.0	30.5	110 " " "
" 31	70	0.424	29.68	1,089	20.7	30.5	30 " " " "
Feb. 1	68	0.380	25.84	948	18.2	30.3	
" 4	80	0.312	24.96	916	18.1	29.5	Diarrhea.
" 5	60	0.395	23.70	870	14.6	29.0	" Noon meal omitted.
" 6	48	0.317	15 21	558	8.4	29.5	
" 7	74	0.213	15 76	578	14.1	29.0	
" 8	47	0.307	14.42	529	11.5	28.5	
Average.....			21.63	794	15.2		Diet 1.0 gm. of nitrogen per kilo.

and ate all food. The bile pigment figures are included and show remarkable and inexplicable fluctuation, particularly with the beef heart diet.

The taurocholic acid output is quite uniform each day with occasional fluctuations. The average daily output of bile acid per 6 hour period shows a very remarkable increase when the dog was suddenly changed from the diet rich in carbohydrate (0.5 gm. of nitrogen per kilo) to the beef heart diet (1.0 gm. of nitrogen per kilo). The increase in bile acid corresponds to the increase in food nitrogen—that is, about 100 per cent increase. The sharp rise in taurocholic acid following the change to a rich protein diet (beef heart) is well shown in both these experiments. The reaction even goes above the average figures of taurocholic acid output on the very first day of rich protein diet.

The second dog (Table XXXVI) shows a general reaction which is in every respect similar to the preceding experiment but for some diarrhea in the beef heart period. Associated with this was some loss of weight and appetite. These abnormal factors, however, did not influence the uniform reaction to the change in food protein. Additional data which confirm these experiments will be found in Paper V of this series.

Tables XXXVII and XXXVIII add some interesting data to that of the preceding experiments. In both these experiments the food nitrogen was decreased to 0.25 gm. per kilo by decreasing the cracker meal and replacing the beef heart with fat. The caloric value was held unchanged at 100 calories per kilo. One dog (Table XXXVII) showed practically no reaction to this change in diet and excreted almost the same amount of taurocholic acid as formerly upon a diet of 0.5 gm. of nitrogen per kilo. The other dog (Table XXXVIII) showed a decided drop in excretion of taurocholic acid but not to one-half the output on the diet containing 0.5 gm. of nitrogen per kilo. The level of bile acid output on this cracker meal-fat diet approaches the fasting excretion level of bile acid. This shows at once that there is no hard and fast parallel between the nitrogen intake and bile acid excretion, but it is clear that there is an important exogenous factor.

The mixed diet periods show a level of bile acid excretion which is below that of the beef heart diet. The elements in the

TABLE XXXVII.

Nitrogen in Food and Bile Acid Excretion.
Dog 17–151. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Feb. 11	32	0.525	16.80	617	38.8	40.0	262 gm. of cracker meal, 10 gm. of butter, 30 gm. of lard, 105 gm. of cane sugar, 0.25 gm. of nitrogen, and 100 calories per kilo.
" 12	30	0.536	16.08	590	28.2	39.5	
" 13	11	0.602	6.62	243	10.4	39.8	Hb. 125 per cent. R. B. C. 6,240,000.
" 14	32	0.425	13.60	499	20.6	40.0	
" 15	24	0.527	12.66	465	20.9	40.0	
" 18	32	0.385	12.32	452	10.0	40.0	No bile exclusion in this experiment.
" 19	20	0.493	9.86	362	13.2	40.0	
" 20	45	0.345	15.52	570	14.9		
" 21	23	0.695	15.98	586	5.2		
Average.....			13.27	487	18.0		Diet 0.25 gm. of nitrogen per kilo.
Mar. 5	61	0.534	32.55	1,196	16.6	42.5	Mixed diet.
" 6	48	0.662	31.79	1,167	21.5	41.8	
" 7	80	0.290	23.19	852	36.9	42.5	Hb. 118 per cent. R. B. C. 6,350,000.
" 8	32	0.695	22.25	818	10.1	43.0	
" 11	38	0.286	10.86	399	21.9	41.5	
" 12	30	0.771	23.14	850	3.9	43.0	
" 13	28	0.786	22.00	809	11.3	42.5	
" 14	40	0.616	24.64	906	5.8	43.5	
" 15	22	0.691	15.20	559	12.6	44.0	
Average.....			22.85	839	15.6		Mixed diet.

TABLE XXXVIII.

Nitrogen in Food and Bile Acid Excretion.
Dog 18–23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Feb. 11	48	0.231	11.08	407	18.4	30.3	30 gm. of lard, 59 gm. of cane sugar, 198 gm. of cracker meal, 10 gm. of butter, 0.25 gm. of nitrogen, and 100 calories per kilo.
" 12	43	0.174	7.47	274	16.1	29.5	
" 13	68	0.100	6.80	253	16.6	29.5	Hb. 115 per cent. R. B. C. 7,928,000.
" 14	58	0.027	1.60	58	18.2	29.3	
" 15	63	0.129	8.10	297	18.4	29.3	No bile exclusion in this experiment.
" 18	35	0.114	3.97	145	13.4	29.0	
" 19	64	0.197	12.64	464	11.1	29.2	
" 20	55	0.172	9.48	348	19.1		
" 21	51	0.085	4.34	159			
Average.....			7.27	267	16.4		Diet 0.25 gm. of nitrogen per kilo.
Mar. 5	75	0.241	18.06	664	31.8	32.0	Mixed diet.
" 6	64	0.249	15.93	585	36.3		
" 7	85	0.173	14.73	541	37.0	33.3	Hb. 110 per cent. R. B. C. 6,290,000.
" 8	81	0.210	17.04	626	37.5	34.0	
" 11	91	0.200	18.22	670	27.9	32.8	
" 12	69	0.184	12.72	467	29.0	34.0	
" 13	82	0.324	26.60	978	25.3	35.0	
" 14	60	0.300	18.01	662	27.6	35.5	
" 15	82	0.307	25.14	923	29.5	34.8	
Average.....			18.50	679	31.3		Mixed diet.

mixed diet are of course variable. This diet is a mixture of kitchen scraps—bones, meat, bread, potato, rice, etc.

Table XXXIX shows a third dog which was placed upon these same diets containing 0.5, 1.0, and 0.25 gm. of nitrogen per kilo. This dog remained in perfect condition during the entire experiment and adds confirmatory data to the other experiments. This bile fistula dog has been under observation for 3 years, and it is known that there is a small communication between the common duct and duodenum. This allows a small amount of bile to enter the duodenum when the fistula is not draining freely, for example at night. This dog shows only a 50 per cent rise in bile acid output when the diet is changed from 0.5 to 1.0 gm. of nitrogen per kilo. Further change in diet from 1.0 to 0.25 gm. of nitrogen per kilo causes a fall from 1,262 mg. of taurocholic acid to 684 mg. per 6 hour period. These fluctuations are not proportional to the nitrogen content of the diets, but it is obvious that a rise in the food nitrogen intake does cause a rise in the taurocholic acid excretion and *vice versa.*

Table XL gives the data on two experiments which show the influence of a preceding period of fasting upon subsequent excretion of bile acids with a standard diet of 0.5 gm. of nitrogen per kilo. It is to be recalled that these same two dogs on a previous occasion showed an output of bile acids on this same diet which was much higher (550 and 875 mg. of taurocholic acid per 6 hour period—Tables XXXV and XXXIX). Compare with this high output the low excretion in Table XL on the same diet (376 and 419 mg. of taurocholic acid per 6 hour period). The only factor which can explain this difference is the preceding fasting period of 10 and 11 days. This low level of excretion is actually that of the fasting period or even lower. It is evident that the fasting period has caused a changed reaction in the body so that much less bile acid is permitted to escape in the bile. It may be a correct assumption that some of the material which under usual diet conditions goes to form the bile acid fraction is deviated for other uses in the body.

This reaction must be kept in mind whenever any diet experiments are planned for these bile fistula dogs. For certain experiments it might be assumed that a preliminary period of fasting might give an ideal simple base line from which to estimate the change brought about by a given diet. But the reaction may be

TABLE XXXIX.

Nitrogen in Food and Bile Acid Excretion.

Dog 15–22. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Jan. 7	13	0.835	10.85	398	10.0	32.0	325 gm. of cracker meal, 75 gm. of beef heart. 100 calories and 0.5 gm. of nitrogen per kilo.
" 8	20	0.992	19.84	728	3.5	32.3	
" 9	32	0.662	21.18	778	3.3	32.3	
" 10	26	0.591	15.36	551	3.6	32.5	No bile exclusion in this experiment.
" 11	38	0.373	14.17	520	7.3	32.3	
" 14	35	1.071	37.48	1,375	8.5	32.3	Large amount not eaten.
" 15	34	0.751	25.53	936	4.1	32.0	100 gm. not eaten.
" 16	14	0.920	12.88	473	3.4	32.0	100 " " "
" 17	46	0.630	28.98	1,065	6.4	32.5	150 " " "
" 18	43	0.822	35.35	1,297	7.5	32.0	
" 21	60	0.759	45.54	1,670	4.2	32.3	25 " " "
" 22	42	0.453	19.00	698	9.5	32.0	
Average.....			23.84	875	5.9		Diet 0.5 gm. of nitrogen per kilo.
Jan. 23	38	0.915	34.77	1,277	5.3	32.0	571 gm. of beef heart containing 100 calories and 1.0 gm. of nitrogen per kilo.
" 24	45	0.750	33.75	1,238	8.6	31.5	
" 25	62	0.700	43.40	1,593	5.4	31.0	
" 28	35	0.566	19.81	727	24.2	30.5	Diarrhea.
" 29	22	1.400	30.80	1,130	10.3	30.8	
" 30	30	1.730	51.90	1,905	7.8	31.0	
" 31	46	1.100	50.60	1,855	11.2	30.8	
Feb. 1	28	1.394	29.03	1,065	6.8	30.8	
" 4	29	1.131	32.80	1,205	13.6	31.0	
" 5	39	1.015	39.58	1,453	6.0	30.8	Noon meal omitted.
" 6	12	1.896	22.75	835	6.3	31.0	
" 7	42	0.698	29.31	1,075	6.6	30.5	
" 8	29	0.980	28.42	1,042	11.6	30.3	
Average.....			34.37	1,262	9.5		Diet 1.0 gm. of nitrogen per kilo.

TABLE XXXIX—*Concluded.*

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Feb. 11	29	1.105	32.05	1,175	11.6	32.0	209 gm. of cracker meal, 10 gm. of butter, 30 gm. of lard, 65 gm. of cane sugar, 0.25 gm. of nitrogen and 100 calories per kilo.
" 12	23	0.654	15.04	550	16.0	31.3	
" 13	47	0.403	18.95	696	8.4	32.0	
" 14	23	0.633	14.56	534	36.0	31.5	Hb. 124 per cent. R. B. C. 5,632,000.
" 15	48	0.658	31.58	1,160	10.8	31.8	
" 18	45	0.296	13.32	488	24.3	30.5	
" 19	28	0.331	9.26	340	14.6	30.5	
" 20	12	1.410	16.92	620	13.0	31.0	
" 21	17	0.922	14.97	599	17.2	31.3	
Average.....			18.50	684	16.8		Diet 0.25 gm. of nitrogen per kilo.

very different toward the same diet factor depending upon whether a fasting period or a carbohydrate diet period had preceded. To get a complete understanding of a single diet factor it will be necessary to observe any change in the bile acid excretion curve which may be associated with the administration of any such substance after short fasting periods as well as after carbohydrate or high protein diet periods.

Table XLI gives data to indicate that the formation of bile acids depends in part upon the functional capacity of the liver. The Eck fistula liver is produced by an anastomosis between the portal vein and vena cava and a ligature on the portal vein above this anastomosis which limits the blood supply of the liver to the hepatic artery and deviates all of the portal blood directly into the vena cava. The Eck fistula liver is known to be smaller than normal, to exhibit a moderate degree of fatty degeneration, to show a marked decrease in production of bile pigments (Whipple and Hooper, 4), and to present a distinct impairment of its normal capacity to

TABLE XL.

Nitrogen in Food and Bile Acid Excretion after Fasting Period.

Dog No.	Date.	Volume.	Amino nitrogen. In 1 cc. of bile.	Amino nitrogen. In 6 hours.	Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
17–151	Average 10 days fasting.....			11.8	433			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
	May 4	37	0.454	16.80	616	11.0	32.6	Diet 330 gm. of cracker meal, 75 gm. of beef heart, containing 0.5 gm. of nitrogen and 100 calories per kilo.
	" 6	19	0.349	6.64	224	1.6	36.25	
	" 7	20				2.4	35.5	
	" 8	27	0.367	9.91	364	3.8	35.8	Hb. 130 per cent. R. B. C. 7,235,000.
	" 9	25	0.350	8.75	321	6.1	34.7	
	" 10	24	0.407	9.77	359	6.7	35.0	
Average.....				10.37	376			Diet 0.5 gm. of nitrogen per kilo.
15–22	Average 11 days fasting.....			11.09	407			
	May 6	21	0.587	12.32	452	2.9	29.2	Diet 285 gm. of cracker meal, 60 gm. of beef heart, containing 0.5 gm. of nitrogen and 100 calories per kilo.
	" 7	32				5.6	29.5	
	" 8	36	0.353	12.70	466	9.4	29.2	Hb. 125 per cent. R. B. C. 7,335,000.
	" 9	29	0.365	10.58	388	6.1	29.0	
	" 10	24	0.421	10.10	371	4.2	29.2	
Average.....				11.42	419			Diet 0.5 gm. of nitrogen per kilo.

excrete phenoltetrachlorphthalein (Whipple, Peightal, and Clark, 5). We may assume that the Eck fistula liver is functionally deficient. The observations in Table XLI harmonize with those

TABLE XLI.

Nitrogen in Food and Bile Acid Excretion. Eck Fistula. Dog 16–15. Simple Bile and Eck Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Jan. 7	16	0.314	5.03	183	4.5	20.5	Diet 200 gm. of cracker meal, 55 gm. of beef heart, containing 0.5 gm. of nitrogen and 100 calories per kilo.
" 8	25	0.258	6.45	236	5.7	20.0	Hb. 99 per cent. R. B. C. 6,240,000.
" 10	12	0.219	2.62	96	1.9	19.8	Not eating well.
" 11	16	0.144	2.30	84	5.3	19.8	" " "
" 14	12	0.155	1.86	68	3.3	20.0	
" 15	6	0.116	0.69	25		20.2	
" 16	10	0.141	1.41	52	1.6	20.7	
" 17	14	0.330	4.62	169	3.2	19.8	
" 18	21	0.273	5.73	210	3.7	20.3	50 gm. not eaten.
" 21	20	0.310	6.20	227	2.1	20.0	
" 22	20	0.212	4.24	155	2.1	20.0	
Average.....			3.74	137	3.34		Diet 0.5 gm. of nitrogen per kilo.
Jan. 24	30	0.447	13.41	492	3.7	19.8	Diet 355 gm. of beef heart, containing 1.0 gm. of nitrogen and 100 calories per kilo. 1 gm. of yeast twice a day.
" 25	17	0.534	9.07	333	0	20.8	

outlined above and show that an Eck fistula dog upon a standard diet will excrete not over one-half the normal amount of bile acids.

Compare this dog (Table XLI), weight 20 lbs. and output on standard diet of 137 mg. of taurocholic acid per 6 hour period, with the dog of Table XXXV, weight 40 lbs. and output on the same

standard diet of 550 mg. of taurocholic acid per 6 hour period. Also compare with dog of Table XXXIX, weight 33 lbs. and output on the same standard diet of 875 mg. of taurocholic acid per 6 hour period.

When the Eck fistula dog was changed to a rich protein diet we observe a considerable jump in the output of bile acids, as in the other dogs. It is not possible to keep an Eck fistula dog on a pure beef heart diet for any length of time without precipitating the characteristic Eck fistula intoxication which usually results in death.

DISCUSSION.

There is sufficient experimental data given above to make the point that both endogenous and exogenous factors are concerned in the metabolism of bile acids. There is a reasonably constant output of taurocholic acid during fasting periods, and this output may be somewhat diminished by administration of pure carbohydrate. The relative diminution of urinary nitrogen and taurocholic acid excretion may show a certain similarity under these experimental conditions. This may indicate a certain relationship between the metabolism of the body protein and the production of taurocholic acid. There is obviously a very important endogenous factor in the metabolism of bile acids.

It is equally clear that there is an important relationship between the output of taurocholic acid and the intake of certain food proteins. On certain diets a uniform level of bile acid excretion may be observed for days and a sudden shift to a similar diet containing double the amount of food nitrogen may cause a sudden doubling of bile acid excretion. This fact comes out clearly in several experiments in this paper but we wish to refer to additional evidence submitted in Paper V of this series. It is certain that some food proteins act very differently from others as to their value in modifying the bile acid excretion.

It is to be noted that the increased excretion of bile acids appears very promptly when the diet is changed to beef heart. It may be that the formation of bile acids by the liver cell is an automatic response to the proper stimulus, just as these cells respond to a protein digestion stimulus by urea formation. In the case of urea formation we know that even in the greatest food

shortage emergency the liver will form urea from amino-acids, even though the body may need all the food amino-acids, for example, after long periods of fasting. In the case of taurocholic acid we have shown that long periods of fasting followed by high protein feeding will show little rise in the taurocholic acid output contrary to what we might expect if this substance was purely a waste product to be eliminated from the split products of protein digestion. The body evidently conserves the taurocholic acid or its parent substance under certain conditions, perhaps for use elsewhere in the body in the reconstruction of its depleted body protein.

It is generally accepted that bile acids are dependent upon the normal liver cell function for their production. There has been little dispute in medical literature concerning this point. Yet there is very little available direct proof of this statement if one wished to argue that the bile acids were formed elsewhere in the body and eliminated in the bile. For example there is convincing evidence that bile pigments may be formed outside of the liver and excreted in the bile secondarily (Whipple and Hooper).

We have evidence in the Eck fistula experiment given above that bile acid output will be much subnormal in the Eck fistula liver which is functionally subnormal. This indicates that in a general way the bile acid output may fall with impaired functional capacity of the liver cell. Other experiments in which the liver is injured by chloroform and other poisons give similar results which will be published in the near future. All this evidence gives some direct and positive proof that the bile acids are actually produced by liver cell activity.

SUMMARY.

There is a uniform excretion of taurocholic acid in the bile fistula dog during fasting periods.

There is a uniform and slightly lower excretion of taurocholic acid in the same dog during similar periods of carbohydrate (sugar) feeding. This fall in taurocholic acid excretion is much like the fall in urinary nitrogen excretion under the same conditions.

There is an important endogenous factor in the bile acid metabolism and this may concern the body protein metabolism.

The output of bile acid in bile fistula bile may be influenced at will by suitable control of the diet. Meat protein seems to be of the greatest importance and a pure meat diet will give the highest output of bile acid per 6 hour period.

There is an important exogenous factor in bile acid metabolism which is concerned especially with the food protein.

After a long fasting period the bile fistula dog will not react to a high protein diet with the usual high bile acid output. There is evidently a deviation of certain precursors of the bile acid to serve other purposes in the body—perhaps to supply some important substances relating to body protein which have been depleted by the fasting period.

A functionally deficient liver (Eck fistula) produces less than one-half the normal amount of bile acid during a standard diet period. This is direct evidence (of which there has been little available) that the bile acids are formed essentially by liver cell activity.

BIBLIOGRAPHY.

1. Bidder, F., and Schmidt, C., Die Verdauungssäfte u. der Stoffevechsel, Mitau, 1852.
2. Kunkel, A., *Arch. ges. Physiol.*, 1877, xiv, 344.
3. Spiro, P., *Arch. Physiol.*, 1880, Suppl. 50.
4. Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1917, xlii, 544.
5. Whipple, G. H., Peightal, T. C., and Clark, A. H., *Johns Hopkins Hosp. Bull.*, 1913, xxiv, 343.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE METABOLISM OF BILE ACIDS

V. CONTROL OF BILE INGESTION AND FOOD FACTORS

BY

M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM
THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOL. XXXVIII, No. 2, JUNE, 1919

Reprinted from The Journal of Biological Chemistry, Vol. XXXVIII, No. 2, 1919

THE METABOLISM OF BILE ACIDS.

V. CONTROL OF BILE INGESTION AND FOOD FACTORS.

By M. G. FOSTER, C. W. HOOPER, and G. H. WHIPPLE.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, April 28, 1919.)

The experiments tabulated below serve two purposes. They furnish additional data to make absolutely certain that the amount of bile acid excreted can be increased with the increase of meat protein nitrogen in the diet. These experiments in addition show that absolute exclusion of every drop of bile by mouth does not modify the excretion of bile acids under the conditions of the experiment. It was necessary to submit these control experiments because Stadelmann, who has done exceptional experimental work in this field, has stated that bile exclusion will cause a fall of bile acid excretion. He assumes therefore that a muzzle at night is necessary for accurate work, and that the bile fistula dogs lick sufficient bile from their fistulas to modify the following day's excretion of bile and bile acids.

Our dogs are set up each morning after a period of exercise followed by a 30 minute period of free drainage before collections are started. After the collection period of 6 hours the dogs are allowed to exercise in the yard before being fed in their cages. They are kept during the night in large cages of wire mesh beneath which are pans for the collection of excreta. The dogs, of course, during the night drain bile from their fistulas into the pans, but no bile collects where the dogs have access to it because the wire mesh retains no fluids. The dogs usually lick their fistulas when the collection is finished but it seems to be due in part to the fact that the skin itches where it is in contact with the abdominal binder. These dogs rarely lick bile from their fistulas in the early morning and at this time the flow is at a minimum. We feel that the experiments given make it quite clear that under

this laboratory routine the amount of bile which may be obtained by a given dog licking its own fistula is not sufficient to modify the excretion curve of bile acids.

We cannot attempt to explain Stadelmann's results, but two factors may be concerned. It is well known that dogs which have had bile fistulas for many months or years do not lick their fistulas as much as do dogs who are less accustomed to the bile fistula. One of our dogs has been under observation with a bile fistula for 3½ years. Furthermore, in our cages, as stated above, bile could not accumulate during the night where the dog had access to it at any time, as the bile flowed through the mesh cage floor. If bile accumulated on the floor of a room or cage it is very probable that a dog would lick some of this bile in the morning and obtain a sufficient amount to make a decided difference in the output of bile acids.

It can be deduced from the experiments given in Paper III that moderate amounts of bile given late in the afternoon will not influence the output of bile acids on the following day. Bile given in the forenoon will cause a cholagogue action lasting several hours, but usually a rapid elimination of the excess of bile acids within 6 hours—at least 80 to 90 per cent elimination within this time.

EXPERIMENTAL.

Bile exclusion in these experiments indicates that the dog in question was unable to gain access at any time to any bile either from its own fistula or elsewhere. During the routine 6 hour collection all the bile is collected in a small rubber bag. At the end of the collection a muzzle is put securely on the dog, which is then permitted to run in the yard for a few minutes under careful observation. The dog is then brought in and dressed with its night binder which is worn until the next morning. The muzzle was not worn during the night except in one instance, where the dog tried to chew off the straps of the abdominal binder. The night binder was made of light canvas and fitted to the individual to cover the thorax and abdomen completely. Anteriorly it was held from slipping backward by soft webbing which encircles the neck and fore legs. The binder was held about the abdomen by soft webbing or straps. A large gauze pad was

placed over the bile fistula and served to absorb all the night bile. With this routine we are absolutely certain that no bile was ingested at any time during periods of "bile exclusion." The animals were comfortable and maintained their usual conditions of diet and activity.

Tables XLII, XLIII, and XLIV are to be considered as a unit. These three experiments were done at the same time under identical conditions, and the results are remarkably uniform. They are to be compared with Tables XXXV, XXXVI, and XXXIX in Paper IV of this series. In each of the three experiments tabulated (XLII to XLIV) the dog was placed upon a diet of cracker meal and beef heart containing 0.5 gm. of nitrogen and 100 calories per kilo. This diet with complete bile exclusion obtained for 1 week, and the average daily output per 6 hours shows a fairly uniform figure of 400 to 500 mg. of taurocholic acid per 6 hour period. The dog which is slightly heavier shows a slightly greater output.

The three dogs kept on this same diet for the second week were not prevented from licking their own fistulas in the cage during the night. It will be seen that the bile acid output remains about the same—it is actually somewhat less during this second week, 340 to 440 mg. of taurocholic acid per 6 hours.

During the third week each dog was given the first day with no bile exclusion, the second and third day with complete bile exclusion, and the fourth day with no bile exclusion. Individual fluctuations appear but this week in general agrees with the data of the first 2 weeks.

The fourth week was continued with strict bile exclusion but the diet was changed to a mixture of beef heart and a little cracker meal, giving 1.0 gm. of nitrogen and 100 calories per kilo. The figures from this group of experiments resemble those referred to in Paper IV. The increase in beef heart stimulates the output of bile acids from a level of 400–500 mg. to 700–900 mg. per 6 hours.

Two points are made by these and other experiments. Bile exclusion does not modify the excretion of bile acids under the conditions of our experiments. Certain food proteins in the diet have a marked influence on the excretion of bile acids in bile fistula bile.

TABLE XLII.

Known Diet With and Without Bile Exclusion.
Dog 18–93. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.			
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Aug. 5	57±	0.256	14.59±	536±*	31.8	Absolute bile exclusion. Diet 330 gm. of cracker meal. 60 gm. of beef heart = 0.5 gm. of nitrogen and 100 calories per kilo.
" 6	46	0.320	14.70	540	31.5	
" 7	38±	0.254	9.66±	355±*	32.0	
" 8	56	0.238	13.33	489	32.0	
" 9	59	0.255	15.05	552	32.3	
Average.....			13.50	494		Complete bile exclusion.
Aug. 12	54	0.224	12.08	443	30.8	No bile exclusion.
" 13	61	0.210	12.81	470	31.5	
" 14	58±	0.242	14.05±	516±*	31.5	
" 15	61	0.158	9.64	354	31.3	
" 16	64	0.181	11.58	425	31.3	
Average.....			12.00	442		No bile exclusion.
Aug. 19	70	0.266	18.62	683	31.0	No bile exclusion.
" 20	71	0.196	13.90	510	30.5	Bile exclusion 12 hours previously.
" 21	64	0.230	14.72	540	30.8	Bile exclusion.
" 23	77	0.185	14.25	523	30.5	No bile exclusion.
Aug. 28	72	0.326	23.48	862	30.0	Absolute bile exclusion. Diet 467 gm. of beef heart, 100 gm. of cracker meal = 1.0 gm. of nitrogen and 100 calories per kilo.
" 29	72	0.293	21.08	774	29.8	
" 30	76	0.335	25.48	934	29.5	Hb. 126 per cent. R. B. C. 6,430,000.
Average.....			23.30	857		Complete bile exclusion.

* 2–3 cc. of bile lost.

TABLE XLIII.

Known Diet With and Without Bile Exclusion.
Dog 18–54. Bile Fistula and Splenectomy.

Date.	Volume.	Amino nitrogen. In 1 cc. of bile.	Amino nitrogen. In 6 hours.	Taurocholic acid in 6 hours.	Weight.	Remarks.
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Aug. 5	65	0.170	11.05	405	27.5	Absolute bile exclusion. Diet 280 gm. of cracker meal, 60 gm. of beef heart = 0.5 gm. of nitrogen and 100 calories per kilo.
" 6	62	0.181	11.23	412	27.8	
" 7	41	0.100	4.10	150	28.0	Binder chewed off night of the 6th.
" 8	47	0.182	8.56	314	27.5	
" 9	79	0.212	16.75	614	27.0	
Average.....			10.33	379		Complete bile exclusion.
Aug. 12	77±	0.224	17.25±	633	27.3	No bile exclusion. 5 hour collection.
" 13	51	0.140	7.14	262	27.3	
" 14	43	0.172	7.40	271	27.0	
" 15	57	0.115	6.56	241	27.0	
" 16	53	0.126	6.68	245	27.5	
Average.....			9.00	330		No bile exclusion.
Aug. 19	77	0.222	17.10	628	27.5	No bile exclusion.
" 20	76	0.167	12.68	465	27.3	Bile exclusion 12 hrs. previously.
" 21	71	0.187	13.26	486	27.5	Bile exclusion.
" 23	80	0.128	10.24	376	27.3	No bile exclusion.
Aug. 27	46				27.0	Absolute bile exclusion. Diet 417 gm. of beef heart and 100 gm. cracker meal = 1.0 gm. of nitrogen and 100 calories per kilo.
" 28	81	0.288	23.31	856	26.3	
" 29	75	0.311	23.32	856	26.5	
" 30	70	0.380	26.60	976	26.3	Hb. 110 per cent. R. B. C. 5,515,000.
Average.....			24.40	896		Complete bile exclusion.

TABLE XLIV.

Known Diet With and Without Bile Exclusion.
Dog 18–137. Bile Fistula and Splenectomy.

Date.	Volume.	Amino nitrogen.		Tauro-cholic acid in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.			
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
Aug. 5	53	0.313	16.58	608	23.0	Absolute bile exclusion. Diet 245 gm. of cracker meal, 45 gm. of beef heart = 0.5 gm. of nitrogen and 100 calories per kilo.
" 6	56	0.264	14.78	542	23.0	
" 7	38	0.365	13.86	509	23.3	
" 8	37	0.250	9.25	340	23.0	
" 9	34	0.198	6.73	247	23.0	
Average.....			12.25	450		Complete bile exclusion.
Aug. 12	56	0.196	10.96	402	22.8	No bile exclusion.
" 13	46	0.266	12.24	449	22.3	
" 14	40	0.214	8.56	314	22.0	
" 15	43	0.129	5.50	202	22.0	
" 16	44	0.198	8.71	320	22.0	
Average.....			9.20	338		No bile exclusion.
Aug. 19	50	0.404	20.20	742	22.0	3 hour collection.
" 20	57	0.292	16.65	611	22.3	Bile exclusion 12 hours previously.
" 21	68	0.188	12.78		22.3	Bile exclusion.
" 23	40	0.099	3.96	145	22.3	No bile exclusion.
Aug. 28					21.8	Absolute bile exclusion. Diet 327 gm. of beef heart and 100 gm. of cracker meal = 1.0 gm. of nitrogen and 100 calories per kilo.
" 29	50	0.335	16.75	611	22.0	
" 30	57	0.376	21.42	786	22.0	Hb. 120 per cent. R. B. C. 5,060,000.
Average.....			19.07	698		Complete bile exclusion.

Tables XLV and XLVI supply more data on bile exclusion and fasting excretion of bile acids. These tables are to be compared with Tables XXXII and XXXIV in Paper IV and the average of these last two tables is added to Tables XLV and XLVI now under consideration. Table XLV shows practically the same output of bile acids per 6 hours whether bile is excluded or not. This is in harmony with all the other experiments given in this and other papers as well as many experiments which are unpublished.

TABLE XLV.

Bile Exclusion—Fasting.
Dog 17–151. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
May 27	28	0.693	19.40	712	26.8	41.2	Hb. 120 per cent. R. B. C. 7,520,000.
" 28	24	0.541	12.98	476	23.8		
" 29	19	0.492	9.35	343	9.3	39.0	
" 30	20	0.558	11.20	411	9.0	38.0	
" 31	19	0.653	12.41	455	13.9	37.1	
June 1	23	0.694	15.96	586	10.0	36.5	
Average 6 days ..			13.55	497			Complete bile exclusion.
Average 10 days ..			11.42	419			No bile exclusion. Table XXXII.

Table XLVI presents a single contradiction to all the rest of our published data and taken by itself would seem to indicate that bile exclusion did actually diminish the bile acid output. Such contradictions are not unheard of in experimental work, but we believe this exception to the general rule is in reality explicable. This dog as stated above (Paper IV) is known to have a small opening from his biliary tract into the duodenum. At times he presents remarkable variations in the output of bile acids which stand in contrast to the other dogs whose biliary passages are absolutely separated from the duodenum. Because of this fact we attach little importance to observations on this dog

when the bile is concentrated and viscid, and still less importance to observations which differ from those made upon the other fistula dogs.

TABLE XLVI.

Bile Exclusion—Fasting.
Dog 15–22. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Bile pigments in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.				
1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*lbs.*	
May 27	27	0.905	24.30*	892*	3.7	34.5	Hb. 130 per cent. R. B. C. 6,540,000.
" 29	12	0.532	6.38	234	14.6	32.3	
" 30	21	0.435	9.14	335	18.3	31.5	
" 31	16	0.380	6.08	223	9.7	30.9	
June 1	12	0.562	6.74	247	14.6	30.4	
" 2	20	0.086	1.72	63	46.0	29.7	NH_2 determination repeated with same result.
" 3	16	0.510	8.16	299	24.5	29.4	
Average 6 days ...			6.37	234			Complete bile exclusion.
Average 9 days ...			11.09	407			No bile exclusion. Table XXXIV.

* Not included in average.

SUMMARY.

Complete exclusion of bile ingestion at all times does not modify the excretion of bile acids from day to day. This statement applies to the dogs used in our experiments and to the laboratory conditions under which all this work was done. It is not necessary therefore to take elaborate precautions to prevent any given dog from licking its fistula during afternoon and night resting periods.

These experiments confirm those tabulated in the preceding paper to show that an increase in the meat proteins in the diet will cause a considerable rise in output of taurocholic acid.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THE METABOLISM OF BILE ACIDS

VI. ORIGIN OF TAUROCHOLIC ACID

BY

M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE

(FROM THE GEORGE WILLIAMS HOOPER FOUNDATION FOR MEDICAL RESEARCH, UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL, SAN FRANCISCO)

REPRINTED FROM

THE JOURNAL OF BIOLOGICAL CHEMISTRY

VOL. XXXVIII, NO. 2, JUNE, 1919

Reprinted from THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. XXXVIII, No. 2, 1919.

THE METABOLISM OF BILE ACIDS.

VI. ORIGIN OF TAUROCHOLIC ACID.

BY M. G. FOSTER, C. W. HOOPER, AND G. H. WHIPPLE.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, April 28, 1919.)

Taurocholic acid can be easily separated into its two essential constituents, taurine and cholic acid. It has been shown (Paper III) that in the body the two constituent parts can be easily and rapidly united to form taurocholic acid. This holds true whether the two substances are given by mouth or whether cholic acid is given by mouth and taurine intravenously. It is clear that cholic acid and taurine have a strong physiological attraction in the normal body.

Taurine appears to be present in the normal body in a certain excess, judging from cholic acid feeding experiments. Taurine is more abundant in the body, at least more accessible for linkage with cholic acid, during full diet periods than during fasting periods (Paper III). We have also submitted evidence to show that taurine is derived at least in part from the cystine of the food. This is in harmony with the observations of von Bergman (2) and Wohlgemuth (14), who used somewhat inaccurate methods of analysis. It is highly probable that taurine may be derived from other substances under certain conditions but we have no direct proof concerning this point. When the metabolism of taurine is fully understood we may be nearer a complete understanding of the complex bile acid metabolism.

Cholic acid is a substance which defies the investigator, who up to the present time has learned little about its source or usefulness in the body. Cholic acid is of particular interest to us because its supply appears to be the normal limiting factor which determines the bile acid level of excretion. A sufficient supply of taurine in the body seems to obtain under all physiological conditions. There is sufficient evidence to show the important relationship that

exists between the basal nitrogen metabolism and bile acid metabolism. We believe that knowledge concerning the bile acid metabolism will be of value for a complete understanding of the fundamental body protein metabolism.

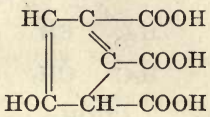
Cholic acid is somewhat complex and its accepted formula is given below. Because of its structural formula many hypotheses have been advanced concerning its origin in the body and its possible relationship to various drugs. Some positive experiments have been reported to indicate a relationship between cholic acid and cholesterol, but these data will not bear careful scrutiny. In the experiments given below we have tested a number of such hypotheses and without exception can report only negative results. It seemed to us of some importance to establish beyond a doubt that cholesterol and allied substances have no influence whatever on the cholic acid metabolism.

Through the work of von Bergman in 1904, and Wohlgemuth in the same year, it was clearly established that some of the taurine of the taurocholic acid came from cystine. Both used the sulfur method in their experiments. Friedman (4) in 1903 had shown that this change could be effected *in vitro*, but von Bergman was the first to show that when cystine is fed to a dog together with sodium cholate on a constant diet, there is a marked increase in the sulfur of the bile. There was a distinct but less striking increase when sodium cholate alone was given by mouth. There was no increase in bile sulfur when cystine alone was fed. Wohlgemuth followed the sulfur of the urine in cats before and after administering cystine. There was a large amount of neutral sulfur excreted, and, as there was no cystine in the urine, he concluded that all the cystine had been changed into taurine. This reasoning may be open to certain objections.

Gibson (5) gave three injections of bromobenzol to cats at 8 to 10 hour intervals to deflect the cystine from its usual cycle and found a marked decrease in the alcohol-soluble sulfur of the bile. He does not mention whether the cats were kept upon a uniform diet nor does he state their clinical condition after administering the drug. The cats were sacrificed 5 to 8 hours after the last injection. We know that this decrease in bile sulfur might have been caused by a diet low in meat protein nitrogen, by refusal of food, or by the intoxication from the bromobenzol.

Lifschütz (8) found that cholic acid gives the same color changes and spectrum as cholesterol or oxycholesterol when treated with acetic acid, benzoylsuperoxide, and sulfuric acid. Flury (3) states that the acids obtained by the oxidation of cholesterol may be placed in the pharmacological group of bile acids and saponins from their pharmacological behavior. He thinks that they may occur as intermediate steps in the formation of bile acids from cholesterol.

Excellent work has been reported by Schrötter, Weitzenböck, and Witt (11, 12). They found that cholesterol, cholic acid, oil of turpentine, and camphor all give the same compound, rhizocholic acid, when treated with concentrated sulfuric acid, mercury, and nitric acid. Rhizocholic acid is:



and was identified carefully in each case. From this fact they conclude that cholic acid and cholesterol belong to the terpene series.

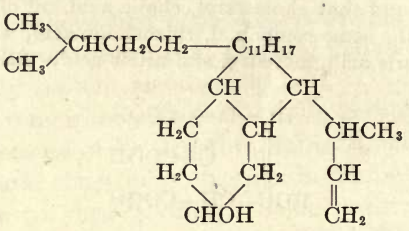
Pregl (10) states that cholic acid is closely related to turpentine and camphor and is a hexahydroxylbenzene. Moreschi (9) in 1913 attempted to show the relation of cholic acid to cholesterol by making a trichlor derivative from each. The cholic acid formed a compound with 11 atoms of chlorine, and cholesterol with 10 chlorine atoms.

Hammarsten (7) states that in certain animals (shark) the bile acids are replaced by scymnol sulfuric acid, made up of a substance he calls "scymnol" which is allied to cholic acid or cholesterol paired with sulfuric acid.

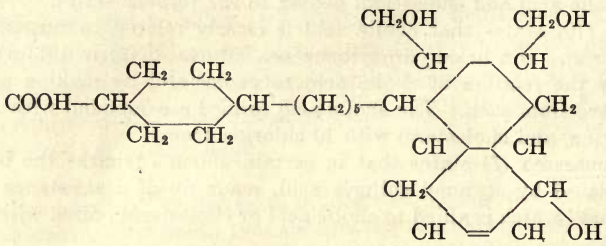
D'Amato (1) found that dogs fed on a lipoid diet gradually increased the excretion of both cholesterol and bile acids in the bile. The increase noted in bile acids is very small and within the limits of error of the method employed and surely within the physiological variations which are observed so frequently in routine collection periods.

Goodman (6) thought that the broken down red blood corpuscles might be available for the formation of bile constituents; *i.e.*, the cholesterol might be the mother substance of cholic acid. He used a method in which he weighed the cholic acid excreted. The diet consisted of dog biscuit (10 gm. of nitrogen per day), varied with red blood cells, egg white, coagulated horse serum, calves' brains, and cholic acid. The cholic acid caused a marked increase in the cholic acid content of the bile, but the bile cholesterol was unchanged. This cholic acid increase might have been due to cholic acid excreted as such, and not to taurocholic acid, for his method determines any cholic acid dissolved in the bile, whether united with taurine or not. The egg albumin increased the cholic acid but not the cholesterol. He used but one dog, and that dog lived only 4 weeks. For this reason and because of inaccuracies in his methods we do not attach much importance to his results. Because there is such a small amount of cholesterol present in the blood cells, Goodman says that in a human being there would be necessary about 60 per cent blood destruction per day, and even more in the dog, which of course is unthinkable. This theory at best could explain only a small part of the actual cholic acid production. Our experiments give no support to this suggestion.

According to Windaus (13), the formula for cholesterol is as follows:



Pregl gives the following formula for cholic acid:—



EXPERIMENTAL.

The experiments were conducted under conditions similar to those described above. The dogs were set up for ½ hour for free drainage of the bile fistula before the collections were started. Only a part of the experimental data is given, but it is uniform in character and the evidence all points the same way.

Table L presents two similar experiments in which cystine was given intravenously with no positive influence on the bile acid output, but with a decided effect upon the bile unhydrolyzed amino nitrogen fraction. This may indicate an excretion in the bile of cystine or taurine under these conditions. On the following day a similar injection of cystine followed by cholic acid by stomach resulted in a great increase in taurocholic acid excretion and a return of the unhydrolyzed amino nitrogen to normal. This gives evidence that under these conditions the body can change cystine rapidly to taurine, which is then available to combine with the cholic acid. It may be objected that on a mixed diet the feeding of cholic acid will increase the output of taurocholic acid because plenty of taurine is available from the food. Refer to Table XXIV, Paper III, where it is seen that the rise in tauro-

cholic acid does actually reach a higher level after administration of 4.0 gm. of cholic acid with a full mixed diet. It would be desirable to repeat these observations during a fasting period.

Taken together with the experiments of von Bergman and Wohlgemuth we feel reasonably secure in the statement that cystine can produce taurine under physiological conditions, and

TABLE L.

Cystine Intravenously Reacts with Cholic Acid.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
15–22	Nov. 25	53	0.227	12.04	442	33.3	Mixed diet.
	" 26	28	0.456*	12.75	468	33.0	0.7 gm. of cystine intravenously.
	" 27	51	0.746	38.05	1,395	32.5	0.7 gm. of cystine intravenously + 2.1 gm. of cholic acid by stomach.
17–34	Nov. 25	35	0.198	6.93	254	30.3	Mixed diet.
	" 26	40	0.356*	14.24	522	30.3	0.7 gm. of cystine intravenously. Vomited.
	" 27	76	0.453	34.45	1,264	30.3	0.7 gm. of cystine intravenously + 2.1 gm. of cholic acid by stomach. Vomited a little of cholic acid solution.

* The *unhydrolyzed* bile gave a high *unhydrolyzed* NH_2 showing the presence of an excess of cystine or taurine uncombined with cholic acid. The following day when cholic acid was given this *unhydrolyzed* NH_2 had returned to normal which would indicate that all the cystine had been synthesized into taurocholic acid.

this taurine is available to combine with an excess of cholic acid. This holds good apparently whether the cystine is fed by mouth (von Bergman) or given intravenously (Table L).

In comparing Table LI with Tables XXXI and XXXIII of Paper IV, one can see that these variations are within the normal fluctuations of these same dogs on a sugar diet.

Tables LI and LII are to be considered together and they furnish strong evidence that *cholesterol* alone or fed with taurine exerts no influence upon the excretion of taurocholic acid. This holds for periods of fasting as well as for periods of mixed diet. The amounts of cholesterol administered (3 to 4 gm.) are sufficient to convince any investigator that this substance does not play a part in bile acid metabolism. There is no immediate reaction nor any

TABLE LI.

Cholesterol Feeding—Sugar Diet—Bile Acids Unchanged.

Dog No.	Date.	Volume.	Amino nitrogen. In 1 cc. of bile.	Amino nitrogen. In 6 hours.	Taurocholic acid in 6 hours.	Pigments in 6 hours.	Urinary N in 24 hours.	Weight.	Remarks.
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*	*gm.*	*lbs.*	
17–151	Average 11 days.			8.05	295		3.45		Diet of 75 gm. of cane sugar, 100 gm. of glucose.
	Apr. 6	15	0.402	6.03	220		2.52	35.0	
	" 7	17	0.556	9.45	347	18.6	2.13	35.1	4 gm. of cholesterol.*
	" 8	17	0.416	7.08	260	14.2	2.57	34.4	
18–23	Average 11 days.			8.66	318		3.23		Diet of 75 gm. of cane sugar, 75 gm. of glucose.
	Apr. 6	37	0.171	6.32	232	14.2	3.30	25.8	
	" 7	31	0.269	9.34	343	13.0	2.58	25.7	4 gm. of cholesterol.*
	" 8	37	0.125	4.62	169	18.7	2.18	25.7	

* Given in gelatin capsule at beginning of 6 hour collection.

delayed effect to be observed. In Table LII the taurine was given to insure an excess of this substance in the body to combine with any amount of cholic acid available. Table LII illustrates the inhibition of bile flow due to sugar by mouth (Dog 15–22). Vomiting on certain occasions may be associated with a decreased flow of bile in these fistula dogs. The amount of ether used to dissolve the cholesterol (8 to 10 cc.) will have no influence upon the bile excretion.

Tables LIII and LIV are similar experiments which show that red blood cells by mouth have no effect upon the excretion of bile acids. The control periods of 5 days and the red blood cell feeding periods give figures which are practically identical. The

TABLE LII.

Taurine Plus Cholesterol Feeding—Bile Acids Unchanged.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
							Mixed diet.
18–93	Sept. 11	76	0.210	15.95	586	31.5	
	" 12	58	0.320	18.55	681	32.5	1 gm. of taurine,* 3 gm. of cholesterol. Some diarrhea. Intoxicated by ether.
	" 13	76	0.225	17.10	628	34.0	Hb. 126 per cent. R. B. C. 6,430,000.
15–22				6.40	235		Average 10 days fasting.
	June 6	3.6	0.642	2.31	84	28.2	100 gm. of cane sugar and 25 gm. of glucose after 10 days fasting with bile exclusion. Vomiting.
	" 7	10	0.616	6.16	226	27.4	2 gm. of taurine,* 3 gm. of cholesterol.
							Mixed diet.
18–54	Sept. 3	42	0.170	7.14	262	27.0	Hb. 110 per cent. R. B. C. 5,515,000.
	" 4	46	0.141	6.49	238	27.4	1 gm. of taurine* and 3 gm. of cholesterol. Intoxicated by ether.
	" 5	58	0.113	6.56	241	28.0	

* Taurine dissolved in water. Cholesterol dissolved in ether and given by stomach tube at beginning of 6 hour collection.

mixed diet gives considerable daily fluctuation, but the averages are nearly uniform. The addition of 10 gm. of dried red blood cells does not influence the curve of bile acid excretion. This amount of red cells contains an appreciable amount of cholesterol, but of course not comparable to the large amounts used in Tables

LI and LII. We have other experiments with brain feeding which show the same negative influence on bile acid excretion.

Table LV shows the negative reaction following the intravenous injection of laked red blood cells. This again illustrates how easily we may dissociate the excretion curves of bile pigments and

TABLE LIII.

Red Blood Cell Feeding—Bile Acids Unchanged.
Dog 18-23. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.			
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
July 15	56	0.272	15.23	559	31.8	Mixed diet. Absolute bile exclusion.
" 16	63	0.254	16.00	587	33.3	Hb. 115 per cent. R. B. C. 6,200,000.
" 17	67	0.297	19.90	731	32.5	
" 18	71	0.410	29.10	1,068	32.0	
" 19	62	0.324	20.18	741	32.3	
Average.....			20.00	734		
July 22	53	0.254	13.47	495	31.0	Mixed diet + 10 gm. of R. B. C.*
" 23	60	0.262	15.72	576	32.3	" " + 10 " " "
" 24	57	0.302	17.20	628	32.5	" " + 10 " " "
" 25	64	0.271	17.35	637	32.8	" " + 10 " " "
" 26	62	0.326	20.20	742		" " + 10 " " "
Average.....			16.80	617		

* Prepared by washing red blood cells in normal saline 3 times. The residual cells were dried by warm air and ground to a powder, 10 gm. of red cells made into an indefinite solution emulsion mixture, flavored with a little sugar and salt, made up to 400 cc. with water, and given by stomach tube.

bile acids. The intravenous injection of large amounts of laked red blood cells will cause a prompt and large increase in the output of bile pigments but no increase in bile acid excretion. There is no immediate and no delayed bile acid reaction which we have been able to observe. Some investigators have recorded a drop in bile acid excretion following intravenous injections of hemoglobin but our experiments are clear cut and negative in this respect. One

observes only the normal physiological fluctuations. A possible explanation of the observed depression of bile acid excretion is the febrile reaction which sometimes is observed following hemoglobin injections. No such reaction was observed in these experiments.

It is well to note that the whole laked cells were given—that is, stroma and hemoglobin. After the blood had been laked by dis-

TABLE LIV.

Red Blood Cell Feeding—Bile Acids Unchanged.
Dog 18–54. Bile Fistula and Splenectomy.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.			
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
July 22	37	0.268	9.91	363	28.0	Mixed diet. Bile exclusion.
" 23	41	0.213	8.71	319	28.3	
" 24	18	0.315	5.67	208	27.8	
" 25	33	0.299	9.87	362	27.5	
" 26	45	0.282	12.68	466		
Average.....			9.40	345		
July 30	65	0.125	8.13	299	28.0	Hb. 100 per cent. R. B. C. 5,075,000. Mixed diet + 10 gm. of R. B. C.*
" 31	48	0.237	11.36	417	28.3	" " + 10 " " "
Aug. 1	50	0.284	14.20	522	27.8	" " + 10 " " "
" 2	37	0.145	5.36	196	27.5	" " + 10 " " " Tube out. About 10 cc. lost.
Average.....			9.7	356		

* Red blood cells prepared as in Table LIII.

tilled water it was shaken thoroughly to insure a complete injection of stroma and cell fragments. It has been suggested repeatedly that the red cell stroma and perhaps the contained cholesterol were waste products which normally came to the liver to be transformed into bile acids and eliminated in the bile. This is an attractive hypothesis which is delightfully simple, but like many others it has no basis of experimental fact and should be put aside even if with regret.

Since Schrötter, Weitzenböck, and Witt were able to make rhizocholic acid from cholic acid, cholesterol, turpentine, or camphor, we thought it might be possible to produce cholic acid in the animal body by feeding either turpentine or camphor. Taurine was given on the last day in each experiment (Table LVI) so that any cholic acid which might be formed in the organism would unite with the taurine and be excreted in the bile as tauro-

TABLE LV.

Laked Red Cells Intravenously—Bile Acids Unchanged.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
18–23	July 29	66	0.262	17.28	637	30.5	Mixed diet.
	" 30	72	0.223	16.05	590	32.3	Laked blood.*
	" 31	58	0.349	20.25	744	32.3	Hb. 120 per cent. R. B. C. 6,375,000.
	Aug. 1	63	0.287	18.18	678	31.8	Laked blood.*
	" 2	51	0.413	21.03	772	32.3	
18–93	July 29	68	0.207	14.07	517	30.3	Mixed diet.
	" 30	66	0.241	15.90	584	31.8	Laked blood.*
	" 31	53	0.280	14.85	545	32.3	Hb. 135 per cent. R. B. C. 6,380,000.
	Aug. 1	70	0.229	16.02	588	32.3	Laked blood.*
	" 2	64	0.315	20.15	740	33.3	

* 100 cc. of sterile defibrinated normal blood, centrifuged, red cells washed and laked with water, made up to 100 cc., and kept on ice over night; rendered isotonic, warmed, shaken, and given intravenously by hypodermic needle 2 hours after start of collection.

cholic acid. This was probably an unnecessary precaution when we recall the great output of taurocholic acid which follows a cholic acid feeding during periods of mixed diet. This indicates an abundant source of taurine in the body during periods of liberal feeding, more than enough to combine with any expected excess of cholic acid.

Careful scrutiny of Table LVI reveals no fluctuations other than those observed in control periods, whether terpene hydrate alone or combined with taurine was given by stomach. There is no

TABLE LVI.

Terpene Hydrate With and Without Taurine by Mouth.

Dog No.	Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
			In 1 cc. of bile.	In 6 hours.			
	1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
							Mixed diet.
15–22	Sept. 3	22	1.150	25.30	929	33.3	Hb. 150 per cent. R. B. C. 7,455,000.
	" 4	47	0.214	10.05	369	34.5	1 gm. of taurine, 1 gm. of terpene hydrate.*
	" 5	45	0.381	17.15	630	33.5	1 gm. of taurine, 1 gm. of terpene hydrate.*
	" 6	38	0.388	14.73	541	32.8	
							Mixed diet.
18–23	Aug. 27	57	0.261	14.86	545	30.8	
	" 28	57	0.343	19.55	718	31.8	0.25 gm. of terpene hydrate.*
	" 29	70	0.309	21.63	794	33.3	0.5 gm. of terpene hydrate.* 0.5 gm. of taurine.
	" 30	53	0.434	23.00	844	33.3	
							Mixed diet.
18–54	Sept. 10	61	0.112	6.84	751	28.8	
	" 11	82	0.238	19.50	716	29.0	
	" 12	81	0.186	15.05	552	28.5	1 gm. of terpene hydrate,* 1 gm. of taurine by stomach tube. Diarrhea.
	" 13	47	0.155	7.29	267	27.8	
							Mixed diet.
17–181	Sept. 10	49	0.292	14.30	525	25.0	
	" 11	42	0.280	11.75	431	25.3	
	" 12	67	0.267	17.85	655	26.0	1 gm. of taurine, 1 gm. of terpene hydrate.* Diarrhea.
	" 13	42	0.324	13.60	499	25.0	
							Mixed diet.
18–93	Sept. 3	64	0.285	18.25	670	30.5	Hb. 126 per cent. R. B. C. 6,430,000.
	" 4	44	0.073	3.94	144	33.5	1 gm. of taurine and 1 gm. of terpene hydrate.* Diuresis.
	" 5	54	0.183	9.89	363	32.5	1 gm. of taurine, 1 gm. of terpene hydrate.*
	" 6	64	0.291	18.61	684		

* Terpene hydrate dissolved in a little dilute alcohol; taurine dissolved in water, given by stomach tube at beginning of 6 hour collection.

cholagogue action and no change in the bile concentration and total amounts of bile acids.

Table LVII shows the results of administration of camphor by mouth. Repeated doses of spirits of camphor have no effect upon the bile acid excretion. Similar observations have been made on

TABLE LVII.

Camphor Feeding With and Without Taurine by Mouth.
Dog 15–22. Simple Bile Fistula.

Date.	Volume.	Amino nitrogen.		Taurocholic acid in 6 hours.	Weight.	Remarks.
		In 1 cc. of bile.	In 6 hours.			
1918	*cc.*	*mg.*	*mg.*	*mg.*	*lbs.*	
July 29	48	0.290	13.92	511	33.5	Mixed diet.
" 30	29	0.155	4.49	165	34.3	0.2 gm. of camphor* by stomach tube. Hb. 135 per cent. R. B. C. 6,430,000.
" 31	35	0.292	10.22	375	34.3	0.4 gm. of camphor by stomach tube.
Aug. 1	38	0.430	16.34	600	33.5	0.6 gm. of camphor by stomach tube. Considerable salivation.
" 2	14	1.030	14.41	529	33.5	
" 5	47				33.8	0.4 gm. of camphor by stomach tube.
" 6					35.0	0.4 gm. of camphor by stomach tube.
" 7	53	0.269	14.25	523	34.3	0.4 gm. of camphor by stomach tube.
" 8	63	0.222	14.00	514	33.3	0.4 gm. of camphor and 1 gm. taurine by stomach tube.
" 9	40	0.439	17.56	644	34.0	0.4 gm. of camphor by stomach tube.

* Camphor given as a 10 per cent solution—spirits of camphor. Diuresis was uniformly noted.

other dogs. Obviously there is no immediate and no delayed reaction which can be attributed to the action of this drug.

It is evident that neither camphor nor terpene hydrate have any effect on the excretion of taurocholic acid. Cholesterol, turpentine, cholic acid, and camphor may all belong to the same group chemically, but physiologically they certainly are not closely related, at least as regards bile acid metabolism.

SUMMARY.

Taurine as found in the body is derived in all probability from the cystine of the food or body protein. Taurine appears to be present in excess of the amount needed to combine with the cholic acid of normal metabolism.

Cholic acid is the limiting factor which determines the level of bile acid excretion in the bile. The origin and fate of cholic acid have not been satisfactorily determined.

Cholesterol fed alone or combined with taurine causes no change in the excretion of bile acids. This gives no evidence of any physiological relationship between cholesterol and cholic acid.

Red blood cells fed by mouth or hemolyzed and injected intravenously have no influence upon the level of bile acid excretion.

Terpene hydrate and camphor fed alone or combined with taurine do not influence the curve of bile acid excretion.

BIBLIOGRAPHY.

1. d'Amato, L., *Biochem. Z.*, 1915, lxix, 217.
2. von Bergman, G., *Beitr. chem. Physiol. u. Path.*, 1904, iv, 192.
3. Flury, F., *Arch. Exp. Path. u. Pharm.*, 1911, lxvi, 221.
4. Friedman, E., *Beitr. chem. Physiol. u. Path.*, 1903, iii, 25.
5. Gibson, R. B., *J. Biol. Chem.*, 1909, vi, p. xvi.
6. Goodman, E. H., *Beitr. chem. Physiol. u. Path.*, 1907, ix, 91.
7. Hammarsten, O., *Z. physiol. Chem.*, 1898, xxiv, 322.
8. Lifschütz, J., *Ber. chem. Ges.*, 1914, xlvii, 1459.
9. Moreschi, *Redn. soc. chim. ital.*, 1913, v, 242.
10. Pregl, F., *Z. physiol. Chem.*, 1910, lxv, 157.
11. Schrötter, H., Weitzenböck, R., and Witt, R., *Monatshr. Chem.*, 1908, xxix, 245.
12. Schrötter, H., and Weitzenböck, R., *Monatschr. Chem.*, 1908, xxix, 395.
13. Windaus, A., Uber Cholesterin; Habilitationsschrift, Freiburg i. Br. Speyer u. Kärner, 1903–Sep. v. Vf.
    Abstracted in *Chem. Zentr.*, 1903, i, 814.
14. Wohlgemuth, J., *Z. physiol. Chem.*, 1903–04, xl, 81.

THE WAVERLY PRESS
BALTIMORE, U. S. A.

THIS BOOK IS DUE ON THE LAST DATE STAMPED BELOW

AN INITIAL FINE OF 25 CENTS WILL BE ASSESSED FOR FAILURE TO RETURN THIS BOOK ON THE DATE DUE. THE PENALTY WILL INCREASE TO 50 CENTS ON THE FOURTH DAY AND TO $1.00 ON THE SEVENTH DAY OVERDUE.

Biology Library

MAR 8 1933
MAR 20 1935
OCT 1 1937
OCT 14 1937
APR 10 1939
JAN 25 1943
APR 26 1948
DEC 29 1948
NOV 13 1950 DEC
AUG 6 1952
APR 13 1953

JUN 2 1962
MAY 20 1962
DEC 6 1964
29 Nov'64 XY
MAR 22 1973
MAR 14 1973 19

LD 21-50m-1,'3

U.C. BERKELEY LIBRARIES
C026173083

463544
QP1
F6
BIOLOGY
LIBRARY
G
UNIVERSITY OF CALIFORNIA LIBRARY